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Contents

PAGE

SEROLOGIC RELATIONSHIP AMONG THE DERMATOPHYTES, *William B. Sharp* 159

PENETRATION OF RADIOACTIVE IODINE IN THE THYROID GLAND COLLOID, *C. Chagas, E. de Robertis and A. Couceiro* 170

MUSCLE RE-EDUCATION, *Nell Harris Swartz* 179

INVESTIGATIONS ON KERATOCONJUNCTIVITIS IN CATTLE ON THE GULF COAST OF TEXAS, *Joseph J. Reid and Ludwik Anigstein* 187

FIELD INVESTIGATIONS PERTINENT TO BULLIS FEVER. THE LONE STAR TICK, *Amblyomma americanum* (Linnaeus, 1758). Notes and Observations from Camp Bullis, Texas, *James M. Brennan* 204

SOME NEW ASPECTS AND APPROACHES TO THE PROBLEM OF DUST DISEASES, *Carl U. Dernehl and Carl A. Nau* .. 227

AMPUTATIONS IN THE UPPER THIRD OF THE LEG, *G. W. N. Eggers* 238

ORGANIC BEHAVIOR PROBLEMS, *Jack R. Ewalt* 247

PARA-AMINOBENZOIC ACID: ITS ACTION ON EXPERIMENTAL ROCKY MOUNTAIN SPOTTED FEVER, *Ludwik Anigstein and Madero N. Bader* 253

CALLING ATTENTION TO 255

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THE UNIVERSITY OF TEXAS, SCHOOL OF MEDICINE
GALVESTON

SEROLOGIC RELATIONSHIP AMONG THE DERMATOPHYTES

WILLIAM B. SHARP*

Fungi recovered from ringworm lesions were examined for serologic relationship by methods familiar in the differentiation of bacterial groups. A procedure was devised for overcoming certain technical difficulties which had restricted this type of investigation with molds. The study was conceived not only for what it might add to the knowledge of dermatophytes, but also for more information as to whether or not serologic characteristics might serve to screen out etiologically important groups from the assortments of fungi presented in various situations.

The skin flora associated with ringworms is sufficiently well understood for evaluation of such relationships as might be found between antigenic and pathogenic groupings. Interest in these fungi is not restricted to the pathogenicity of certain genera. Recently, antibiotic substances have been demonstrated in relation to dermatophytes (Peck and Hewitt). It is interesting to speculate how the various species may exist in antibiotic equilibrium with each other.

Dermatophytes included in the series were those frequently encountered in Galveston, Texas.† There were 29 dermatophyte strains, from 1 to 6 each of *Microsporum felineum (lanosum)*, *M. audouini*, *M. gypseum*, *Trichophyton mentagrophytes (gypseum)*, *T. rubrum*, *T. epilans (cerebriforme)* and *Epidermophyton floccosum (inguinale)*. There also was 1 strain each of 14 nonpathogenic fungi recovered from the skin about the ringworm lesions. These were saprophytic species of as many different genera: aspergillus, fusarium, alternaria, penicillium, hormodendrum, sporotrichum, torula, spondylo-

*From the Department of Bacteriology, The University of Texas Medical Branch, Galveston, Texas. Received for publication January 8, 1945.

†The strains of dermatophytes that were employed were recovered and identified in the mycology laboratory of the Bacteriology Department by Miss Mildred John, and were largely from patients treated in the dermatology clinic, in the School of Medicine of The University of Texas.

cladium, chaetomium, trichoderma, phoma, helminthosporium, syncephalastrum and paecilomyces.

Antigens prepared from each of these strains were tested by precipitin reaction against 6 serums immune to different species of the dermatophytes, a control serum immune to aspergillus and a normal control serum. The precipitin test with fungi first was described in 1902 by Schutze in the case of some saprophytic yeasts and later was found to be specifically differentiative by Balls. Yeast-like pathogens were differentiated in this manner by Stone and Garrod and others. Molds have presented greater difficulties of immunization; the triturated mycelial matter causes fatal emboli if inoculated intravenously and does not readily pass its antigen into solution for test reaction. Serologic studies of molds have been conducted by various investigators despite these difficulties. Citron, for instance, immunized by inoculation into the peritoneum, Conant and Martin by cutaneous injection. Sharp described an antigenic preparation which was tolerated by animals when injected intravenously and was suitable also for serologic reaction *in vitro*.

The antigenic matter utilized for the present experiments was extracted somewhat as described by me in the report just mentioned. The vegetative mat of fungus tissue grown on the surface of the Sabouraud broth was first ground up in 10 per cent sodium chloride solution by ball mill so as to dissolve the protein constituents. After riddance of the fungus pulp, this protein matter was separated by salting out. The residue was freed from most of the inorganic salt and kept in aqueous stock as antigen.

The unit strength of the antigen was gauged during the salting out process. Full strength was regarded as that concentration which remained after the residue had been thrown down but not packed by centrifuge and the supernatant fluid removed. This unit and the dilutions based thereon are largely arbitrary since, from a more closely packed residue as unit strength, the reactive dilutions and titer readings would be correspondingly higher. Rabbits were inoculated intravenously at five-day intervals with the 1:50 dilution in doses

of 3 cc., 6 cc., and 8 cc.; after a two-weeks rest period they received 3 more doses of 8 cc. each. They attained immunity to a homologous titer of between 1,000 and 8,000.

For the test, serum in quantities of 0.05 cc. was pipetted beneath quantities of 0.2 cc. of the serially diluted antigen. Initial readings were made after an hour or so by ring test, then the reagents were mixed and after twenty-four hours another reading was taken from the settled precipitate. This test was not subject to wide fluctuation in its results. Approximately the same readings were obtained on repetition of the test when using identical reagents. In a trial series of 25 such repetitions, 15 tests reacted to identical titer, 8 to the next dilution (half or double) and 2 to the second next dilution.

The reading of a positive reaction and its degree was based on the highest dilution of antigen in which a light but distinct precipitation occurred and on the volume of the precipitate in all dilutions set up. In Tables I, II, and III, the reactions are expressed from zero to 4 plus. Very marked reactions (ordinarily of titer ranging upward from 4,000) were entered as 4 plus; pronounced positives (1,000 or 2,000), or 3 plus; less pronounced but definite positives (250 or 500) as 2 plus; weak positives (125) as 1 plus, and doubtful reactions (only a trace of precipitation up to 125) as 1 plus minus. Negative reactions are those in which there is no trace of precipitation in the 1:125 dilution. Reaction in concentrations up to 1:100 is sometimes spontaneous or otherwise misleading, therefore it was not used. The comparisons in Table IV are expressed by dilution titer.

The existence of common antigens is indicated throughout the entire group of dermatophytes. Greater or lesser degrees of cross-precipitation occurred between all of the various species and nearly all of the strains tested. These reactions are entered in Tables I and II, three-fourths of all tests showing definite precipitation and most of the remainder, a doubtful reaction. These cross-reactions are of differing degree, as will be shown presently. The wide tendency to cross precipitate indicates the serologic interrelationship of these several dermatophytes.

This interpretation derives from the uniform failure of fungi other than dermatophytes to react. Table III shows the tests between dermatophyte immunized serums and the antigens of 14 random fungi of different genera that live saprophytically on the skin. There were occasional doubtful reactions, but apart from these all were negative. The doubtful reaction is one in which there is no precipitation except for a trace in the lowest dilution (125). An animal was immunized to one of these control fungi, *Aspergillus flavus*, and its serum precipitated with the homologous antigen to high titer (4,000). This aspergillus antiserum is seen from Tables I, II, and III to have no precipitative effect with any of the dermatophyte antigens, or with antigen of any of the saprophytes apart from itself.

Strong group reactions have been noted by various observers among closely related species of fungus. Link and Wilcox brought this out in connection with the numerous species of fusarium. Cross-reactions between different members of the genus fusarium proved to be the rule; ordinarily the titers were lower than that with the homologous species, but ranged up to that level and occasionally ran even higher. This broad group relationship among fungi contrasts with the narrow specificity among bacteria. The bacterial group that most resembles the fungi in this, as in some other respects, is the acid fast. The mycobacteria exhibit less serologic than cultural differentiation, according to Wilson and others. Topley and Wilson mention this as a curious anomaly among bacteria—serologic examination being a far more delicate method of differentiation as a rule than the cultural examination.

Comparative titers of the various cross-reactions afford evidence of certain differentiations within the ringworm fungus group. Their interpretation must have due regard, however, for pronounced variations in reactiveness of the individual preparations of antigen and immune serum. Occasional specimens of antigen reacted weakly; for example, that of strain No. 5 reacts more weakly than the other strains of *M. felineum* (Table I). This could result from the crude gauge of unit strength depended upon in preparation. However, this was

not the rule; the several preparations of antigen proved to be fairly uniform in strength and character, regardless of age in storage or preparatory differences. Table IV shows the reactions with antigen preparations from two different cultures of each of 5 strains of dermatophytes. The two antigens selected for each given fungus were made about a year apart; when one was a few weeks old and the other between a year and eighteen months old, the two were tested with the same antiserum. Both homologous and heterologous reactions generally were similar for the two antigens of each fungus. A few individual reactions were in exception to this, notably with the epidermophyton antigens.

More conspicuous fluctuation occurred in the immune level reached by the respective individual serums after the stated course of inoculations. What was even more confusing, these serums did not show the same ratio between homologous and heterologous reactiveness. Those immunized to *E. floccosum* and *T. rubrum* (Tables I and II) attained a degree of immunity only slightly higher than the rest of the serums for the immunizing organisms, but considerably higher for the series of dermatophytes other than those used for their immunization.

So far as can be told from a single experiment it appears that where immunization becomes more intensive, the tendency to precipitate with another species of fungus approaches closer to the homologous level. An animal was immunized to strain No. 2 of *M. felineum*—first with the usual series of inoculations and subsequently with an additional series. Reactions after the first course of injections were similar to those shown in the tables for serum immunized to strain No. 1, pronounced to marked positive for the *M. felineum* and *M. audouini* antigens (titer 2,000 or above) and in most instances weakly positive, doubtful or negative for antigens of the other dermatophytes. After the supplementary course of injections, precipitative titer became little higher for *M. felineum* or *M. audouini* strains, whereas the reactions with other dermatophytes became positive throughout, with titer ranging upward from 250 in most instances and in some cases up to 2,000. Reaction

with the saprophytes remained negative. Link and Wilcox found similarly with fusarium that where the homologous titer has been high and the heterologous low, the titer of the latter was raised more proportionately than that of the former after additional injections of the animal.

After all allowance was made for individual variations in reactiveness of the respective antigens and serums, there remained other differences in titer level which were pronounced and followed a sufficiently definite pattern to establish differentiations within the ringworm group.

First of all, it may be noted that the serum immunized to one strain of any given fungus precipitated to relatively high titer with other strains of that fungus. Observers have long recognized this, and suggestion of it appears in the literature repeatedly. Citron's early experiments indicated that an immunizing species of trichophyton would precipitate better with the immune serum than would another species.

In the case of all the dermatophyte immunizations of my series, as will be noted from Tables I and II, the serum immunized to strain No. 1 of the fungus reacted well with all of the several strains of that same fungus. More often than not these reactions were to a titer of 4,000 or above. In these instances species identification might be established seriologically, since other species did not ever react to so high a titer, except as between *M. felineum* and *M. audouini.*

Pronounced precipitations to lower titer (1,000 or 2,000) would seem to have but presumptive value for identification purposes, and precipitations less pronounced than that would have a suggestive value only. The cross-precipitations between different species of dermatophyte ranged up to those levels. The aforesaid intensive immunization experiment suggests that an antiserum without its immunity forced too high would discriminate the better between the immunizing species and another. Identification would in any event require an extensive series of control reactions. Antigenic means for identifying a dermatophyte seems particularly desirous in the case of strains that have become pleomorphic, where the distinctive microscopic and cultural characteristics have disappeared.

An especially close relationship was indicated between *M. felineum* and *M. audouini* (Table I). Antigens of these two fungi reacted very much alike to both of the immune serums, sometimes indistinguishably. The *M. felineum* strains included some whose antigen reacted with *M. audouini* antiserum to as marked degree as with their own antiserum, and vice versa. All strains of the *M. felineum* did not give a pronounced positive reaction with the *M. audouini* antiserum. Strain No. 5 reacted only weakly to it, but its reactiveness throughout was relatively low. Reaction of the other *M. felineum* strains to *M. audouini* antiserum were stronger and ranged up to very marked positive. About the same may be said of *M. audouini* strains. While the reciprocal reactions between these two species were not always pronounced, they were more frequently so than those between any other two species examined.

These two microsporums were clearly set apart serologically from the remaining dermatophytes of the series. Reaction was of uniformly low degree between serums immunized to them and antigens of the various trichophyton and epidermophyton species (Tables I and II). The same differentiation appears less distinctly in the reactions between antigens of these two and serums immune to the others. The epidermophyton immune serum reacted well with antigens of the two microsporums, as it did with all dermatophyte antigens, having developed unusually broad cross-precipitating potency; but the epidermophyton antigens did not so react with the microsporum immune serums.

The one strain included of *Microsporum gypseum* (Table I) does not react as if closely related to those other microsporums. Its reactions with the several immune serums more closely resemble those of epidermophyton or trichophyton. Although these serologic data accord with the morphologic in approximation of the *M. felineum* with *M. audouini*, they do not accord therewith in bracketing the *M. gypseum* with these other two.

Antigens and antiserums from the strains of *T. mentagrophytes*, *T. rubrum* and *T. epilans* appear from Table II to exhibit a considerable degree of inter-reaction, though a lesser degree than that between *M. felineum* and *M. audouini*. Fur-

thermore, the epidermophyton (Table I) reacts so like trichophyton as to afford little or no evidence of generic distinction. The same may be said of the strain of *M. gypseum* above mentioned, and the strain of unidentified trichophyton. All of these show closer approximation to one another than to the two microsporums first mentioned. Allowing for the varying immunity of the individual serums, the titers suggest an antigenic interrelationship of not greatly dissimilar level throughout.

Within this trichophyton-epidermophyton-M. gypseum group of fungi there appeared no subdivisions with distinctly closer interrelationship than that of the group as a whole. On the other hand, a separation into smaller serologic subgroups is not ruled out; if significance is attached to moderate as distinct from lower titer reactions, there are suggestions thereof. Moderate titer cross-precipitation reaction uniformly occurs between *T. mentagrophytes* and *T. rubrum* strains (Tables I and II). Titer range of such reaction is from 500 to 1,000 or thereabouts. *E. floccosum* antiserum so reacts with *T. epilans* strains more commonly than with the others, but in this instance the suggested closer relationship is not borne out by reactions of the *T. epilans* antiserum. Low titer reactions, ranging up to 500, relate these four together and also the *M. gypseum*. The high titer cross-precipitations that demarcate *M. felineum* and *M. audouini* as a separate closely related group range upward from 1,000.

The serologic data that I have described do not contribute materially to the subclassification of the dermatophytes. The *Microsporums felineum* and *audouini* responsible for the two types of scalp ringworm in children may be said to form one distinct group. Evidence was not obtained of additional groupings of this sort among the ringworm fungi.

Conclusions

1. Dermatophytes of the genera microsporum, trichophyton and epidermophyton are found by the precipitin reaction to have a common antigen, demonstrating interrelationship.

2. Relative titer establishes certain distinctions within the ringworm group, but not adequately for satisfactory subclassification. *M. felineum* and *M. audouini* exhibit close relationship, with clear distinction from the other dermatophytes.

3. Different strains of a given species of dermatophyte react with one another to notably high titer, sufficient to afford aid in identification.

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TABLE I

Reactions Between Microsporum or Epidermophyton and Various Dermatophytes.
Serums immunized to:

Antigens of:	M. felineum strain #1	M. audouini strain #1	T. mentagrophytes strain #1	T. rubrum strain #1	T. epilans strain #1	E. floccosum strain #1	Aspergillus flavus	Normal
M. felineum								
strain #1	++++	++++	++	++	+	++	0	0
strain #2	++++	++++	++	++	++	+++	0	0
strain #3	++++	++	±	++	0	++	0	0
strain #4	+++	++	+	+	+	+	0	0
strain #5	++	+	±	+	0	++	0	0
strain #6	++++	++	+	++	±	+++	0	0
M. audouini								
strain #1	+++	++++	+	+	+	+	±	0
strain #2	+++	+++	±	++	±	++	0	0
strain #3	+++	++	+	++	±	+++	0	0
M. gypseum ...	+	±	±	++	±	++	0	0
E. floccosum								
strain #1	+	0	+	++	+	++++	0	0
strain #2	0	±	+	++	±	++++	0	0
strain #3	±	0	+	++	±	++++	0	0

TABLE II

Reactions Between Trichophyton and Various Dermatophytes.
Serum immunized to:

Antigens of:	M. felineum strain #1	M. audouini strain #1	T. mentagrophytes strain #1	T. rubrum strain #1	T. epilans strain #1	E. floccosum strain #1	Aspergillus flavus	Normal
T. mentagophytes								
strain #1....	+	±	++++	+++	++	++	0	0
strain #2....	+	±	++++	+++	+++	+++	0	0
strain #3....	+	±	+++	+++	+	++	0	0
strain #4....	±	±	+++	+++	+	+++	0	±
strain #5....	±	±	++	++	+	++	0	0
strain #6....	±	0	+++	+++	++	+++	0	0
T. rubrum								
strain #1....	+	±	++	++++	+	++	0	0
strain #2....	±	±	++	++++	+	+++	0	0
strain #3....	+	+	++	++	++	++	0	0
strain #4....	+	+	++	++++	+	+++	0	0
strain #5....	+	0	±	++++	±	++	0	0
T. epilans								
strain #1....	+	±	++	++	+++	+++	0	0
strain #2....	±	±	++	++	+++	+++	0	0
strain #3....	+	±	++	+++	+++	+++	0	0
strain #4....	±	±	++	++	+++	+++	0	0
Trichophyton sp?	±	±	+	++	++	+++	0	0

TABLE III

Reactions Between Dermatophytes and Saprophytes.

Antigens of:	Serum immunized to: M. felineum strain #1	M. audouini strain #1	T. mentagrophytes strain #1	T. rubrum strain #1	T. epilans strain #1	E. floccosum strain #1	Aspergillus flavus	Normal serum
Aspergillus flavus ...	±	0	0	0	0	0	++++	0
Fusarium	0	0	0	0	0	0	0	0
Alternaria	0	0	0	0	0	0	0	0
Penicillium	0	0	0	±	0	0	0	0
Hormodendrum	0	0	±	0	0	0	0	0
Sporotrichum	±	0	±	0	±	±	±	0
Torula	0	0	0	0	0	0	0	0
Spondylocladium ...	0	0	0	0	0	0	0	0
Chaetomium	0	0	0	0	0	0	0	0
Trichoderma	0	0	0	0	0	0	0	0
Phoma	0	±	±	±	0	±	±	0
Helminthosporium ..	0	0	0	0	0	0	0	0
Syncephalastrum ...	0	0	0	0	0	0	0	0
Paecilomyces	0	0	0	0	0	0	0	0

TABLE IV

Effect of Long Storage on the Antigen.

Antigens of:	Serums immunized to: M. felineum strain #1	M. audouini strain #1	T. Mentagrophytes strain #1	T. rubrum strain #1	T. epilans strain #1	E. floccosum strain #1	Aspergillus flavus	Normal serum
M. felineum								
recent antigen..	4000	2000	125	1000	125	125	<125	<125
old antigen	4000	2000	<125	500	250	2000	<125	<125
M. audouini								
recent antigen..	500	1000	125	125	<125	125	<125	<125
old antigen	1000	2000	<125	125	<125	1000	<125	<125
E. epilans								
recent antigen..	250	<125	250	250	2000	1000	<125	<125
old antigen	<125	<125	250	250	250	500	<125	<125
T. rubrum								
recent antigen..	125	<125	500	2000	125	500	<125	<125
old antigen	<125	<125	250	2000	<125	2000	<125	<125
E. floccosum								
recent antigen..	<125	<125	<125	500	<125	8000	<125	<125
old antigen	250	<125	1000	2000	<125	8000	<125	<125

PENETRATION OF RADIOACTIVE IODINE IN THE THYROID GLAND COLLOID*

C. Chagas, E. de Robertis,† and A. Couceiro

The thyroid gland has the specific property of concentrating selectively great quantities of iodine. This capacity has made possible the intensive use of radioactive iodine in order to establish the site and the metabolism of this element in the gland. The localization of iodine in the thyroid has been studied by the autographic technique instituted by Hamilton et al. and by relatively rough separation methods, as the one described by Tatum, which offers no assurance of distinguishing the cellular iodine from the iodine contained in the follicular colloid. Autography has shown the location of organic iodine in the colloid, and recently an attempt has been made to combine the autographic method with the freezing-drying technique, so as to preserve also the inorganic fraction of iodine.

The histologic location by radio-autography is limited, however, by the scattering of radiation in the photographic emulsion; thus, only an approximate distribution of radioiodine is secured, no further quantitative data being obtainable. Consequently, we have sought to obtain better and more direct information on the localization of iodine within the follicle by use of radioiodine, extraction of very small quantities of follicular colloid by micropunction and subsequent radioactive determination with a Geiger-Muller counter.

Experiments have been made *in vivo* with the thyroid glands of rats; here it was possible to study the penetration rate of radioiodine in the follicular colloid in different func-

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†Visiting Research fellow from the Institute of General Anatomy and Embryology, Faculty of Medical Sciences, Buenos Aires; assisted by a grant from the Rockefeller Foundation.

tional states of the glands. It was found that this rate was increased by thyrotropic hormone, and in contrast was inhibited to suppression by thiourea.

Technique

White rats which previously had been given injections of I^{131} in doses varying from 9.1 to 13.8 gamma were anesthetized with nembutal before their thyroid glands were exposed. The observation of the thyroid isthmus was made with a binocular microscope, a low magnifying lens with 100 x power, which permitted the exact delimitation of the follicles. The aspiration of the colloid was done by micro-pipettes which had a bore of 5–100 micra at the tip. These pipettes were introduced into the follicle by the use of a micro-manipulator of Peterfi's type, then suspended in a reversed position above the animal.

Micro-aspiration was performed with a squirt connected to the micro-pipette by a long tube of flexible copper. The liquid jet was controlled by the movement of the aspirator which in turn was governed by a narrow screw. To avoid contamination by interstitial fluid, the surface of the gland was dried by an air jet, as recommended by Wearn and Richards, and the tip of the micro-pipette was filled with mercury. When the pipette was inside the colloid, aspiration was performed and the greatest possible quantity of colloid extracted. The volume of the colloid extracted was measured by determining the diameter of the drop clinging to the tip of the pipette, observed with an ocular micrometer, immediately after extraction. The drop then was placed in tin foil, dried and directly applied to the surface of the Geiger-Muller counter tube. This counter was a glass-walled hydrogen-filled tube, as described by Bale.

We have used the colloid obtained from the aspiration of several follicles in order to get a good number of counts. In this way individual variations of activity were eliminated. The volume obtained has varied between 0.0180 and 0.0749 cm. Radioiodine concentration in the colloid is obtained by comparison with the standard solution. We have used a multivibrator as an external quenching circuit, as previously de-

scribed. Standardization of the experiments was achieved by counting either the standard solution or a volume similar to that of the colloid. Notwithstanding entirely different geometric conditions, results obtained by the two methods are quite similar when allowance is made for differences of concentration. Reflection of beta rays in the tin foil may be responsible for this.

Results

Penetration of I^{131} in the Colloid of Normal Glands

In Table I are shown the results obtained with rats given intra-abdominal injections of radioiodine. The dose injected corresponds approximately to 10 gamma in each 100 Gm. of weight. The penetration has been studied in time intervals of thirty-five minutes to sixty-two hours. The last column indicates the quantity of NaI^* in gammas referred to the standard volume of 0.1 cmm. of colloid. There was penetration of iodine from the first half hour; this was the shortest time for any of the experiments which, as a rule, attain their climax forty hours after the injection. Thus, the only animal observed after sixty-two hours showed a diminution of radioiodine. Two experiments were made at different times on the animals numbered 37 and 39. On another animal, not tabulated, 4 successive experiments were made between thirty minutes and nineteen hours; the results were in close agreement with those given in Table I.

Under the conditions described above, we gave injections of NaI^* simultaneously with 5 units of thyrotropic hormone (antuitrin T). The results listed in Table II may be compared with those given in Table I, where rats were injected with the radio-element but without the simultaneous injection of the hormone. Individual variations were not great enough to overshadow the net influence of the AT in the increase of radioiodine penetration. After six to eighteen hours, the concentrations obtained by use of thyrotropic hormone are comparable to those observed in normal rats after forty hours. At the end of forty hours, maximal concentration occurs but on a much higher level than in the controls.

TABLE I.

Action of Thyrotropic hormone on the penetration of I^{131} in the follicular colloid

Order No.	NaI★ injected in gamma	Time after injection	Volume extracted in mm^3	No. of counts	NaI★ in gamma	NaI★ in gamma per 0.1 mm^3
37	13.8	35′	0.0749	5.5	0.0005060	0.00067
37	13.8	1:10′	0.0430	3.9	0.0003588	0.00083
48A	11.4	1:30′	0.0446	3.1	0.0003782	0.00085
46A	11.4	1:30′	0.0449	6.8	0.0006052	0.00134
45B	11.4	1:30′	0.0363	5.1	0.0004182	0.00115
46E	11.4	6:0′	0.0666	7.8	0.0008112	0.00121
39	13.8	14:0′	0.0570	5.6	0.0007784	0.00136
41N	9.2	24:0′	0.0392	6.0	0.0008160	0.00208
41N	9.2	24:0′	0.0528	12.8	0.0017792	0.00336
50D	11.4	40:0′	0.0454	11.8	0.0020608	0.00453
36	13.8	40:0′	0.0180	8.0	0.0007360	0.00408
39	13.8	62:0′	0.0382	6.6	0.0009174	0.00240

TABLE II

Order No.	NaI★ injected in gamma	Time after injection	Volume extracted in mm^3	No. of counts	NaI★ in gamma	NaI★ in gamma per 0.1 mm^3
43B	19.39	1:30′	0.0570	3.8	0.0010184	0.00178
48B	11.4	1:30′	0.0412	3.9	0.0004758	0.00115
46F	11.4	6:0′	0.0555	9.7	0.0010088	0.00181
46I	11.4	8:0′	0.0298	8.3	0.0009379	0.00314
49B	11.4	9:0′	0.0605	8.0	0.0010320	0.00172
50F	11.4	18:0′	0.0476	13.2	0.0021250	0.00446
50D	11.4	40:0′	0.0462	20.8	0.0033488	0.00727

Table III shows the results obtained with young rats given intraperitoneal injections of 10–15 mg. of thio-urea two hours before the NaI*. Two rats that did not receive the thio-urea are included. Colloid extraction was performed in all of them twenty-four hours after the injection of radioiodine. In these animals, the organic and inorganic radioiodine was determined and the results are included in Table II. It is clearly seen that thio-urea inhibits, to suppression in some cases, the penetration of radioiodine in the follicles of the thyroid gland. This inhibition is in close relation to the drop noticed in the total organic radioiodine contents of the gland, and bears no agreement with the inorganic radioiodine pactum which is very variable.

TABLE III

The combined action of thyrotropic hormone and thio-urea on the penetration of I^{131} in the follicular colloid

Order No.	Thiourea injected	NaI★ injected	Colloid volume extracted in mm^3	No. of counts	NaI★ in gamma	NaI★ in gamma per 0.1 mm^3	Percent of dose injected I Inorg.	I Org.
41C	10 mg.	9.2	0.0235	1.6	0.0002106	0.00089	0.89	10.77
41E	10 mg.	9.2	0.0587	0	0	0	7.02	12.33
41F	10 mg.	9.2	0.0267	0	0	0	5.41	5.31
41A	15 mg.	9.2	0.0406	1.3	0.0001807	0.00044	8.23	2.49
41N	Control	9.2	0.0392	6	0.0008160	0.00208	6.48	24.46
41N'	Control	9.2	0.0528	12.8	0.0017792	0.00336	4.25	34.68

Since the action of thio-urea and thyrotropic hormone have opposite results, we thought it would be interesting to study this antagonistic effect directly. Young rats were given injections of 6 mg. of thio-urea thirty to forty-five minutes before the intraperitoneal injection of 5 units of thyrotropic hormone and 11.4 gamma of NaI*. The results seen in Table IV may be compared with those listed in Table II. In every experiment penetration has been studied ninety minutes after injection of the radioactive substance. Table IV shows unequivocally that the inhibiting action of thio-urea neutralizes the action of the hormone so as to suppress totally the penetration of radio-iodine.

TABLE IV

Order No.	Thiourea injected	NaI★ injected in gamma	Colloid volume extr. in mm^3	No. of counts	NaI★ in gamma	NaI★ in gamma per 0.1 mm^3
46C	6 mg.	11.4	0.386	0	0	0
48C	6 mg.	11.4	0.0417	0.7	0.0000854	0.00020
50C	6 mg.	11.4	0.0331	0	0	0
46A	Control	11.4	0.0449	6.8	0.0006052	0.00134
45B	Control	11.4	0.0363	5.1	0.0004182	0.00115
48A	Control	11.4	0.0446	3.1	0.0003782	0.00085

DISCUSSION

Williams and one of our group used contemporaneously the micro-manipulation technique in the study of thyroid glands *in vivo*. This paper presents an improved technique for micro-aspiration to obtain measurement of the extracted volume, thus

allowing a quantitative determination of radioiodine. The great sensitivity of the Geiger-Muller counter and the minute amounts of material observed make this technique one of the most exact methods in histochemistry. The limit to sensitivity is obtained, in our experiments, by the determination of quantities of NaI^*, close to 2×10^{-11} Gm. contained in a volume of 4×10^{-6} ml. This limit may be lowered by the use of more active samples.

With these doses, penetration into the colloid attained its maximum, in normal animals, in forty hours. These results disagree with those obtained in the total gland by Leblond, who, using similar doses, has shown maximal penetration in eight hours. Our results are close to those obtained with infinitesimal doses of radioiodine as shown by Perlman, Morton and Chaikoff, and Leblond. In this study the maximum was seen in forty-eight hours. This difference might be caused by the fact that the penetration and fixation of iodine within the thyroid cells is much faster than penetration into the follicle; in the former circumstance, it attains the saturation level at the end of eight hours. In the colloid, penetration should be slower and gradual, depending upon cellular activity. The results obtained with thyrotropic hormone and thio-urea will greatly reinforce this assumption. It is well known that thyrotropic hormone produces an increase of iodine fixation by the thyroid gland. Usually the experiments with thyrotropic hormone are made with hyperplastic glands lacking iodine due to repeated injections of thyrotropic hormone. The condition of the gland will be very similar to that produced by the use of diets poor in iodine, and the administration of radioiodine will show a great percentage of fixation. In our experiments we used first those glands which did not lack iodine, and have used, simultaneously, the radio-element and hormone. In this case, iodine penetration was not produced at such a rate as in the hyperplastic glands. These results are in agreement with the ones found by Gersh and Baker who were able to show, by the use of ultra-violet absorption spectroscopy, an increase of iodinization of the thyroglobulin of the follicular colloid under the influence of thyrotropic hormone.

Results obtained by Mackenzie et al., confirmed by Astwood and collaborators, Franklin and Chaikoff, Keston and collaborators and by Couceiro show that many kinds of sulfa drugs, as well as other chemicals such as thio-urea and thiouracyl, may produce hypertrophy of the thyroid gland and inhibition to the secretion of thyroid hormone. Franklin and Chaikoff observed by experiments *in vitro* that the sulfa drugs act directly on the conversion of inorganic idodine to diiodotiosine and thyroxin, and Keston and collaborators have obtained similar results with thio-urea.

Our studies demonstrate that thio-urea may totally inhibit the penetration of iodine in the follicular colloid. This action may be compared to the diminution of organic iodine in the total gland, but it still is more conspicuous. Taking into account that inorganic iodine is not noticeably changed by thio-urea, it seems evident that iodine penetration into the follicle may be considered an active cellular process, and the action of thio-urea be due to enzyme inhibition. Another point in support of this assumption appears from the observation of maximal penetration as shown by our tables compared with previous results. The close agreement of the follicular maxima with the results obtained in the total gland with infinitesimal doses, notwithstanding the use of highly concentrated doses, points to an active process and not to a penetration due to a simple diffusion gradient.

We believe that our results corroborate the conclusions drawn by Franklin and Chaikoff, and prove that the introduction of inorganic radioiodine into the thyroid hormone is an active process, a cellular synthesis and not a simple ionic interchange reaction.

Conclusions

1. A micro-manipulation technique which permits the study of radioiodine penetration in the follicular colloid of thyroid glands is described. This technique is sensitive enough to detect quantities close to 2×10^{-11} Gm. of NaI* contained in volumes of 4×10^{-6} ml. of thyroid colloid.

2. The concentration of radioiodine in the normal follicle increases steadily from the first half hour, and probably attains its maximum in forty hours.

3. Thyrotropic hormone injected simultaneously with NaI* increases radioiodine penetration in the follicle.

4. Thio-urea inhibits or suppresses penetration of radioiodine in the follicle. This inhibition is present even when thio-urea is administered jointly with thyrotropic hormone.

5. It seems that radioiodine penetration in the follicle presents itself not as an ionic interchange but as a true enzymatic synthesis.

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MUSCLE RE-EDUCATION

NELL HARRIS SWARTZ*

Muscle re-education, as employed in a program for the control of motor function or its re-establishment when it has been impaired, is essentially a psychological procedure. Predicated on the concept that mental elaboration precedes motor action, that with a few exceptions of instinctive movements all voluntary muscular function is the result of plan (cerebration), and thus, is a learned behavior, such educational procedure, when combined with measures for the maintenance of physiological potentialities, leads to the utilization of these potentialities to the greatest possible efficiency.

The infant comes into the world able only to suck and to evacuate by defecation and urination. These movements apparently are reflex. They are not the result of learning or plan. The infant cannot, however, inhibit these actions. He cannot focus his eyes, turn his head at will or reach out, but must learn, through trial and error, these and all voluntary functions of the body. By means of the activity of the cerebral cortex a pattern of movement is formed.

This pattern of associated responses is a behavior system which involves reciprocal innervation between paired muscles, coördination of synergist restraints, of group actions and of rhythm. Through use and repetition, as the infant matures, the pattern demands less and less conscious attention and passes, finally, into the reservoir of the unconscious to be used automatically as needed. That increasingly complex motor patterns continue to develop into maturity is evidenced by the additional skills we acquire daily. The degree of mental development seemingly depends on the neuron-association capacity of the brain and to the demands made upon it.

However, interference with any part of the system of associated nerve tracts or fibers which carry the impulses (stimuli) from the stored reservoir (cerebral cortex) to the muscles in-

*From the Physical Therapy Group at Hedgecroft, Houston, Texas.
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volved in the action pattern, can set up a block to the operation of the motor plan. Whether this interference be the result of injury or infection, or whether it originates as inhibition to pain due to intense irritation in an antagonist muscle (the Kenny term, "mental alienation"?) or even from hysteria, it appears clinically as dysfunction or paralysis.

Here, three types of such paralysis may be recognized, flaccid, spastic, and apraxic. Each has its peculiar characteristics and its own etiology, but in each the functioning of the motor plan is inhibited. Hence, in all types, so long as myelinated nerve fibres sufficient to carry the impulse remain, muscle re-education will aid in reducing the inhibition to restore function.

In application, it takes the patient, or better, the pupil, back to the infant level. Through educational technique—explanation and instruction (auditory stimulation), exhibition (visual), goal setting (emotional), manipulation (touch: setting up a proprioceptive knowledge), and finally, practice (habit forming)—the patient approaches the goal of the re-establishment of voluntary motor function.

Particularly in neuro-muscular rehabilitation should one differentiate between the terms muscle re-education and physical therapy. Physical therapy may be considered to be the application of physical means (heat, cold, light, electricity, massage and certain exercises) as a preparation for muscle re-education. Such physical measures are useful for combating the disease processes, especially atrophy, and for maintaining physical potentialities, but they are not to be confused with training.

The theories of treatment and education differ. Treatment is the application of an agency such as surgery, a clinical or a physical procedure, to the patient, and implies passive acceptance by the sick person. Education is essentially a training program in which the results depend ultimately on the pupil's powers of assimilation. Assimilation is active participation rather than passive acceptance. Thus, one might treat an anencephalitic monster, but one could not educate him.

Specifically, one should differentiate in muscle training, between the approach of the therapeutist and that of the

teacher. The efficient therapeutist may of necessity borrow some of the skills of the teacher and the teacher may use those of the therapeutist. While the goal of muscular coördination and effective function may be the same, the technical procedure in therapy differs from that in teaching. Muscular coördination is a learned behavior. A learned behavior can be successfully developed more satisfactorily by educational than by therapeutic methods, unless education is considered to be therapy!

It should be remembered always however, that in these cases of dysfunction one is dealing with pathologic conditions and must therefore coördinate the educational procedure with medical treatment. In making use of all tools, such as psychotherapy, physiotherapy, medicine, surgery, and occupational therapy, to combat disease processes and to promote effective muscular coördination and function, one seeks the rehabilitation of the individual in a physical, mental, and emotional manner, in order to achieve the greatest usefulness and happiness in the life which may be salvaged.

Report of Cases

Case I. Mrs. S., aged 48, was suffering from cerebral thrombosis and hemiplegia of seven weeks' duration. At the time the patient appeared for training she presented a typical picture of "stroke." The left arm hung flail and pendulous from the shoulder, and the fingers were flexed. No function was observed in the hand or forearm. The biceps, still tense, could flex the elbow with assistance. The brachialis, triceps, coracobrachialis and deltoid muscles were normal. The shoulder girdle, especially the trapezius, was being used improperly to swing the flail arm. Here, to all purposes, was paralysis.

To prevent contractures and to facilitate training, the patient was prepared by physical therapy. In this particular case, massage and heat, with passive joint movement were employed.

Since the hand, particularly the thumb, involves the largest area in the cerebral cortex, and since, as in this case, it usually is the member most affected by hemorrhage of this type, we started re-education there. Proceeding carefully, moving the thumb through its arc of movement to set up the goal (a proprioceptive knowledge of what was desired), first flexing then extending, we explained to the patient just how the muscles "worked"; how they were attached to the phalanges of the pollicis and where the "pull" must come in order to move the parts. Further, we explained that for one side, i.e., the extensors, to "pull," the other side,

flexors, must "give out" (relax). Continuing thus, instructing (auditory), illustrating (visual), moving the parts slowly and definitely (touch), extending then flexing, at the same time following with our fingers the path of the desired contraction from the insertion of the muscle being educated up toward its origin, using our touch here as an added stimulus (being careful not to stimulate thus any undesired contraction in other muscles), there was presently a flicker of voluntary movement on the part of the patient.

Following through, the entire upper and less seriously affected lower limb were educated. Within a short time voluntary function was established in all muscles. The function was *very* weak at first, but it was increased slowly and carefully by repeated training and exercise. Exercise with assistance, with gravity, against gravity and against resistance.

In all phases of the program care was taken not to fatigue the patient or the specific part. Synergistic restraints in the fixation muscles were guided. Group muscle action was brought into play and rhythm established, finally carrying over into channels of use, of interest and of occupation (occupational therapy).

By repetition, then, association paths were re-established and the program was continued until the patient was able to carry on correctly in the usual and natural pursuits of her living.

Function returns in some cases without guidance. Persons do learn to handle themselves naturally. But the fact that simply by educational means alone the above mentioned patient was enabled, immediately, to move parts that for seven weeks had been non-functioning, would allow the conclusion that the facilities for function (sufficient nerve fibers) always had been present although unused. How much longer they might have remained unused had the patient been allowed to "pick up movement naturally," that is, through trial and error, cannot be estimated. But in "picking up" such function it is logical to suppose that she would have used some muscles before others, that the stronger parts would have been used first, possibly to the exclusion of those weaker. Again, while function was possible in all muscles, those that were weaker might have remained unused until atrophy had resulted and prevented their utilization. Thus may one account for the incoördination, the substitution and misuse of stronger parts for those that are weaker, as so often is seen in cases of spontaneously returned function.

Case II. F. G., a boy aged 4½, was suffering from cerebral palsy due to injury at birth. There was spasticity and hemiplegia of the right side.

In cases of spastic paralysis resulting from injury at birth, the problem is not only to establish a correct pattern of movement but to correct a faulty one. Since birth the patient has progressed along lines of incorrect motor behavior and uncontrolled responses. The attempt was made, through training, to set aside the learned pattern and substitute a correct one. Success depends on the extent of the injury, the coöperation of the pupil, his comprehension and the teacher's knowledge and teaching skill.

F. G. appeared for training. He was intelligent and alert, but had, through unwise suggestion on the part of one of his parents, set up a resistance to training. He first had to be encouraged to "want to do." He had to be shown concrete goals within his immediate comprehension. A game that entailed knocking a large ball sufficed. The purpose was to enable him to extend the forearm. But F. G. did not know how to extend (stretch out) his arm. In fact, it (the forearm) had been carried in flexion for as long as he could remember.

Here, then, direct instruction was begun. First, manually stretching the arm to full extension (there was no fixed contracture in this case), it was explained how he must "turn loose" the biceps in order to "pull" the triceps to accomplish extension of the forearm. To "turn loose," he was taught to concentrate. He was offered mental pictures of "an old dish rag," and of the floppiest things which could be imagined by him. Patting the part (biceps) softly, the fingers followed a relaxing of contraction from the origin toward the insertion. At last true relaxation was felt in the muscle. This was repeated over and over. The "feel" of relaxation was established firmly before the opposing muscle (triceps) was instructed to contract. Usually these opposites of the overstimulated muscle will be weak. Only through repeated training can their strength be built up and that of the spastic antagonist brought down.

Results are not dramatic in spastic paralysis. It was months before F. G. could reach out and really knock that ball. But eventually he could reach out and knock anything. We saw to it that there were many things he would want to "knock."

Here is reported only a small instance of this child's training. All flexor muscles on his right side, both in the arm and leg, had to be taught to relax; they had to be stretched manually the while to prevent contracture. Their antagonists had to be built up in strength. It may be a long, tedious procedure, but when the pattern is established, the patient will be able to respond in a coördinated manner. In spasticity of this type, however, training must be continued, particularly through the child's growing years. Relaxation of the regime will result definitely in retightening of the muscles, which in turn will

result eventually in contracture and deformity. Even with training, these processes may become established. It is, perhaps, the slowest, most painstaking, most disheartening of all types of paralysis to treat. With the exception of surgical procedure for correction of deformities, or appliances for support, such training remains the only means of accomplishing even partial recovery.

Case II. D. J., a girl, aged 3½, was referred for treatment of poliomyelitis (anterior) fourteen days after onset. There was paralysis of the abdominal muscles and complete involvement of the left lower limb. The back was in spasm (rigid) and the posterior neck muscles were weak.

In this application of muscle re-education, one deals with damage to the anterior horn. The block is in the grey matter of the spinal cord rather than in the brain proper. The principle, however, remains the same. There is interference. Thus, so long as nerve fibers sufficient to carry the stimuli remain, muscle re-education will aid in restoring function. Without an absolute method of determining whether such fibers do remain, we can only presume in every case that they do—until results show otherwise.*

D. J. was treated with Kenny packs on her legs, neck and back for a period of three months (until rigidity receded) to relieve pain and to promote relaxation. Muscle re-education was started as soon as there was sufficient reduction in pain, and was preceded by passive joint movement. Training was begun in this case with the foot—the part most affected. The toes were put through passive movement of extension, one movement at a time here since there was no question of relaxation in an opposing muscle; all muscles in the entire limb appeared to be flaccid.

The child was instructed as to where she must "pull." "See, there are strings tied to the toes—here . . . " Illustration was given by touching the insertions of extensors digitorum. "Now, you know how you pull a string to make a puppet move? Well, you pull right here."

The path of desired contraction upward was followed, from insertion toward the origin of the muscle, moving the toes into extension at the same time with the other hand. This movement was repeated over and over, but only to the point of fatigue. Corresponding parts on the other foot then were used, and illustrated with the undamaged toes, their corresponding movements, using bilateral controls when possible. In three weeks, response was obtained. Immediately, the child was rewarded with praise.

*In a series of 26 cases of poliomyelitis with involvement treated by this method during 1943–44, the following results were recorded: 9 patients recovered completely, 6 made good recovery, but showed some slight after effect (atrophy), 7 showed residual weakness (paresis in one or more muscles and 4 showed residual paralysis in one or more muscles.

One year after onset of the disease there was return of function in all muscles but the anterior tibialis. Paresis, weakness remained in gastrocnemius, soleus and biceps femoris. The child walked with a slight limp but showed steady improvement. Training was being continued. There was slight atrophy in left calf and foot but no deformity.

Case IV. O. C., a boy aged 4½, came for treatment twenty weeks after an accident which resulted in paralysis of the right peroneals, toe extensors and lumbricales with foot drop in the inverted position due to brain injury (concussion). There was also a fracture of the left femur. Upon examination there was revealed the difference of one and one half inches in the length of his legs. The left femur had mended with an overlap. The leg had been set in traction, but because of the serious condition of the patient as a result of the injury to his head, the bone could not be reset. Serious abrasions resulted from the traction splint on the ankle. Paralysis of the muscles of the right foot was diagnosed as a result of the brain injury. The child could not evert his foot or extend or spread his toes. The anterior tibialis showed an inclination toward contracture. The medial longitudinal arch was in slight cavus. To prevent talipes varus, the right foot was fitted with a short caliper brace which held it in a normal position between periods of re-education.

The patient was prepared for training by heat and massage. The left leg was stretched routinely. Re-education of the non-functioning muscles of the foot proceeded, after which general instruction in walking and exercise was given. After a period of four months the following results were recorded: Personeals, extensors and lumbricales (right) are functioning weakly, with assistance, with gravity and weakly against gravity. With instruction the child can walk in a fairly normal manner for a short time without the support of shoe or brace. He shows fair use of the peroneals, but for only short intervals. However, his strength is increasing. Treatment and training are being continued. Present difference in length of leg is three fourths of one inch.

In this case, it would appear that those muscles which had not functioned for a period of weeks were made to function almost immediately, if weakly, upon re-education. Again, this would allow the conclusion that facilities sufficient to obtain function were present but unused until re-education reactivated the motor pattern.

Summary

Given sufficient myelinated nerve fiber to transmit the impulse from the cerebral cortex, function of the muscle awaits a "plan for movement." Hence, education of the muscles, as in any learned behavior, facilitates the origin of such a plan, hastens the course, rules out many mistakes by guiding function along lines of correct anatomical structure and physiology

to make use of every remnant of nerve fiber to the greatest possible muscular efficiency.

Just how this is accomplished—whether through establishment of new paths of association, through detours around injured centers, through neighboring synapses or through remnant paths in the central nervous system—remains to be determined. But for the present it would seem sufficient that such is accomplished when there is a return of function, partial or complete, as in the cases reported and in many others similar to them.

INVESTIGATIONS ON KERATOCONJUNCTIVITIS IN CATTLE ON THE GULF COAST OF TEXAS*

JOSEPH J. REID† AND LUDWIK ANIGSTEIN‡

There is reason to believe that the syndrome, keratoconjunctivitis (pink-eye), in ruminants, although clinically fairly uniform in the description of various authors (conjunctivitis, lacrimation, photophobia, corneal lesions, nasal discharge), is heterogenous from the etiologic standpoint. The analysis by Rose (1942) of his observations on keratitis in cattle of Kentucky shows that in addition to infectious keratitis, clinically identical forms of allergic and nutritional nature (vitamin A deficiency) coexisted in that area. A careful study of this condition due to vitamin A deficiency was made by Schmidt (1941), and later by Jones, Schmidt and associates (1943). These authors produced evidence of eye lesions in cattle which may be confused with infectious keratitis.

The infectious form of the disease which may assume large proportions, not only in cattle but also in other ruminants, was studied in various countries. Some investigators have reported the occurrence of intracellular organisms in the conjunctiva of affected animals. They identified these organisms on morphologic grounds with rickettsiae as causative agents (Coles, 1935; Donatien and Lestoquard, 1939; Mitscherlich, 1943; Rudolf and Zoller, 1943) although the arthropod vector was never established. Little attention was paid in these studies to the possible specific rôle of the bacterial flora of the eye despite the fact that more than twenty years ago the bacterial origin of ophthalmia in cattle was established by Jones and Little (1923). Since that time not much has been added to

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†Texas Agricultural Experiment Station, Substation No. 3, Angleton, Texas.

‡Department of Preventive Medicine, The University of Texas, Medical Branch, Galveston, Texas.

these bacteriologic findings. However, an important advance was made recently in investigations on human keratoconjunctivitis when the specific agent was proved to be a filtrable virus (Sanders, 1942).

In view of the above facts, the etiology of the infectious keratoconjunctivitis in cattle was analyzed in the present study from the rickettsial, viral and bacterial angles.

Distribution and Economic Importance. This disease is widely distributed among range, semi-range and feed lot cattle on the North American Continent, and also has been reported in ruminants from various countries of Europe and Africa. The present work was carried on in the subtropical Gulf Coast area where the disease is prevalent among cattle during all months of the year, but particularly during summer. This highly contagious disease, which has no regard for breed, sex or age, is of vast economic importance to the cattle industry. The vision of the infected animal is impaired or completely destroyed, which naturally prevents it from finding sufficient food, water, and shelter; this results in a considerable loss in body weight—or even death. During the last three years the senior author has had an opportunity to observe a number of outbreaks of the disease among cattle in this area. In Hereford herds the rate of infection is high, being 60 per cent or more. In mixed herds, the Brahman is resistant to some extent; in dairy herds the infection may be light among adults but severe among the calves. Many of the so-called cases of "cancer eye" are a direct sequel of keratoconjunctivitis and such animals usually are condemned when slaughtered for human consumption. Herds in which the infection has made its appearance must be subjected to treatment; such routine brings about an additional loss in body weight and unnecessary financial expense. When these infected animals are offered for sale their owners suffer a financial loss. Nevertheless, the economic importance of this disease has not yet been fully recognized by the cattle industry.

Symptomatology and Pathology. The infection of the eye, as encountered under range conditions, is usually unilateral, but approximately 10 per cent of the cases are bilateral. The

course of the disease is divided into two distinct phases—acute and chronic. In the acute stage one finds a rise in body temperature from 1 to 3 degrees. There is marked lacrimination, conjunctivitis, vascular congestion, photophobia, edematous swelling of the lower lid and viscid mucoid nasal discharge (Fig. 1). The chronic stage begins after a duration of about

Fig. 1. Spontaneous keratoconjunctivitis in cattle. Note the discharge and cloudiness of the cornea.

three days; at this time the pain is very intense, the discharge from the eye has become purulent, the eye lids are swollen and very sensitive. The cornea becomes partially or entirely covered with a whitish film causing opacity. While the chronic stage is in progress it has been observed that ulceration and rupture of the cornea is frequent, which results in complete loss of vision to the eye involved.

From biopsies of the conjunctiva the following pathologic lesions were observed: In acute cases there was comparatively slight inflammatory reaction with edema and hyperemia of the tissue. There was edema and swelling of the covering epithelium. The important findings were the dominating lymphocytes, mononuclear cells and a few plasma cells, while polymorphs were absent. The chronic stage showed a hyperplastic edema with diffuse infiltration which tended to remain on the surface (Fig. 2). Lymphocytes and plasma cells were in-

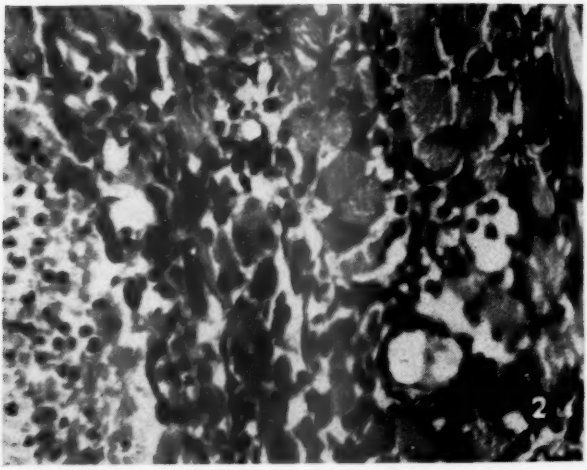

Fig. 2. Eyelid section from chornic keratoconjunctivitis. Diffuse lymphocytic infiltration of the depeer tissue. Giemsa stain. 450 X.

creased in number. The polymorphs appeared between the epithelial cells. Beneath the epithelium and superficial connective tissue there was subacute inflammation, hyperemia and infiltration showing diffuse lymphocytosis. The endothelium of the blood vessels was swollen, but no perivascular infiltration was found. The lymph follicles were hyperplastic and showed very clear germinal centers.

Epizootic factors. Keratoconjunctivitis, or "pink-eye" of cattle, is contagious, being transmitted from one animal to

another by direct contact. In our experimental work no difficulty was encountered in transmitting the disease from an infected to a susceptible animal by rubbing the exudate from the infected eye into the conjunctiva of another calf. It also was found that the nasal exudate was as virulent for susceptible animals as the eye exudate. The period of incubation and the severity of the attack, as determined by experimental inoculation, depends to a great extent upon climatic conditions. The period of incubation is short during the warm months of the year, from forty-eight hours to a week, whereas during the cooler months it may be prolonged to several weeks. Under ordinary range conditions during sunny summer months it is not unusual to find herds in which the rate of infection is from 30 to 70 per cent. When the disease makes its appearance in a herd it spreads with such rapidity that some arthropod vector or vectors were suspected as responsible for the transmission; various fly species have been used in an experimental effort to transmit the disease, but as yet none have been incriminated. According to our observations the incidence of the disease is favored by close contact and therefore is particularly high in crowded herds. Pasturage in this area is confined to the prairies which are almost devoid of shade or shelter. During the summer heat animals seeking shade are obliged to huddle close together and if the disease is present among these animals the rate of infectivity will be high and its spread rapid. Since it was found that the eye and nasal exudates of infected animals were virulent for susceptible animals, it was reasonable to conclude that the disease is spread by licking, rubbing, coughing or sneezing.

Microbiologic findings. Direct examination of smears from the conjunctiva in acute cases which were stained with Giemsa, methylene blue or other stains revealed numerous organisms along with epithelial cells, leucocytes and long strands of mucus. Among the organisms early demonstrated in smears from the exudate of acute cases were coccal and bacillary forms found in abundance; of these, the bacilli deserve, as will be seen later, special attention. In many acute cases they are very numerous and dominate the microscopic field. They stain readily with aniline dyes, are difficult to decolorize after

Gram's method, and usually show a marked tendency toward bipolar staining. They usually appear as diplobacilli with rounded ends and are arranged in groups, clusters or pallisades. In impression smears stained with Giemsa, some epithelial cells contained numerous small coccal and diplococcal bodies within the cytoplasm. They closely resemble the rickettsia-like organisms found in the conjunctival epithelium in opthalmia of goats, as reported by Coles (1935), and in sheep and cattle (Donatien and Lestoquard, 1939).

Since the disease is transmitted from infected to susceptible animals by direct contact, attempts were made to grow the causative agent on artificial media from the exudate. Scrapings were made from the conjunctiva of the infected eye and plated on nutrient agar. The following organisms were isolated: *Escherichia coli*, *bacillus pyogenes*, *bacillus subtilis*, *Pasteurella* and *staphylococcus*. When these organisms were placed on the conjunctiva of normal cattle no evidence of the disease developed. In addition, suitable media consisting of embryonic mouse brain and ox serum ultrafiltrate, described by Sanders (1942), were used in an attempt to cultivate the causative agent. Infected calves, during the acute stages of the disease, were restrained in a squeeze chute and the infected eye was washed by gravity using 1,000 cc. of sterile normal salt solution. The conjunctiva then was scraped with a small sterile curette and tubes of the above medium inoculated. These cultures of mixed bacterial flora were incubated at room temperature for eight days, then ground and instilled into the eyes of susceptible Hereford calves. Out of thirty-five trials made, 10 animals developed the disease. When, however, bacteriologically sterile filtrates of these cultures were injected intracranially into several series of white Swiss mice or instilled into eyes of susceptible calves, no evidence of disease was produced. Several samples of sera from cows at various stages of keratoconjunctivitis and from those convalescent from the disease were sent to Major Murray Sanders, M.C., A.U.S., with the purpose to test these sera against the virus of human keratoconjunctivitis. Since all neutralization tests were negative no relationship between these two diseases exists.

At this point our attention was paid to the possible rickettsial nature of the agent and for this purpose developing eggs were used as a medium. Infectious material was collected in normal salt solution containing 10 per cent ox serum. A portion of this was centrifuged in an angle centrifuge for ten minutes, placed in ice box and allowed to stand for thirty minutes. The supernatant fluid was carefully removed and injected into the yolk sac of five-day fertile eggs; the remaining portion of the material was passed through a fine fritted glass Buechner funnel filter (F) and the filtrate injected into the yolk of five-day fertile eggs. Blind transfers were made at five-day intervals, and several branches carried through to the fiftieth passage. A total of 4,074 eggs were inoculated for transfers. In the course of these operations it was observed that beginning with the fifth egg passage some of the embryos began to die, the fatality rate being irregular and sometimes high. Frequent cultural tests were made for sterility, but invariably with negative results. The dead embryos usually were hemorrhagic, and the egg yolk sac injected. Smears made from the injected yolk sacs fixed with methyl alcohol and stained with Giemsa revealed, in some instances, small coccoid bodies, scattered or in groups. Attempts to infect calves' eyes with this bacteriologically sterile material gave negative results. Therefore, no conclusion could be drawn as to the nature of these bodies.

Isolation of the specific agent. In view of negative results with plain agar or broth, as far as cultivation of pathogenic organisms was concerned, blood agar was introduced. All animals used in this procedure were kept in separate stalls in an insect proof screened barn. On October 25, 1944, eye and nasal exudates from an acute case of the disease were placed on the conjunctiva of both eyes of Hereford calf No. 140. This calf developed a typical keratoconjunctivitis on October 30. Two days later this animal was restrained in a squeeze chute and swabs and scrapings were collected from the conjunctiva and plated on blood agar.

Hemolytic surface colonies appeared on agar plates containing 10 per cent defibrinated rabbit blood and incubated at 36 C. at the end of eighteen hours; these colonies were smooth,

grayish-white and showed a very narrow and clear zone of hemolysis (Fig. 3). At the end of forty-eight hours deep colonies were visible and were characterized by a wide zone of hemolysis. At this time the surface colonies may have reached about 3 mm. in diameter. Examination of these col-

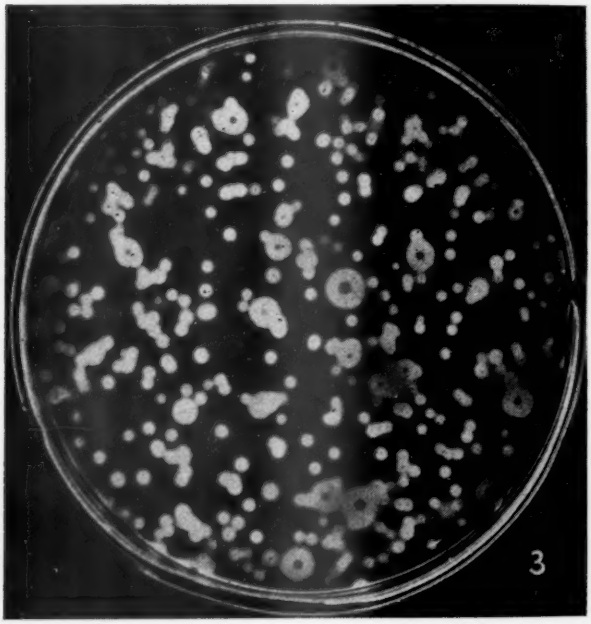

Fig. 3. Twenty-four-hour pure culture of the keratoconjunctivitis diplobacillus on blood agar. Two types of colonies: deep colonies surrounded by a large hemolytic zone and surface colonies showing weak hemolysis.

onies revealed a short, plump diplobacillus with rounded ends, also elongated coccobacilli occurring in clusters and in short chains. Single colony cultures from blood agar slants incubated for forty-eight hours and stained with Giemsa showed the following range of pleomorphism: (1) delicate diplobacilli with rounded ends, a marked tendency toward bipolar staining and occurring in pallisades and short chains, (2) larger bacilli

with rounded ends, sometimes swollen, showing no bipolar staining and sometimes surrounded by a pale extoplasmic layer, (3) single, large, curved thread-like forms showing small endoplasmic granules (Fig. 4). The organism is non-motile

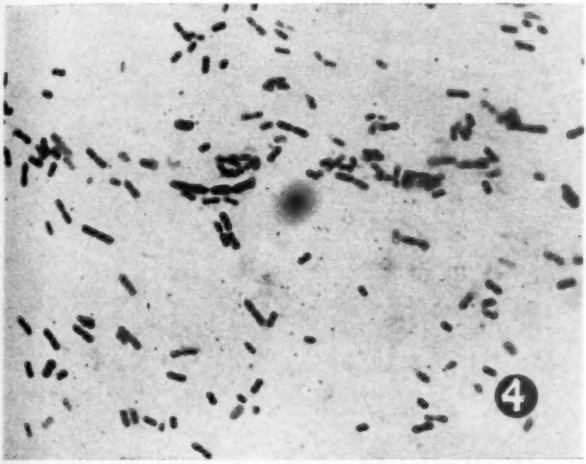

Fig. 4. Smear from blood agar slant 48-hour culture of the keratoconjunctivitis diplobacillus showing the characteristic arrangement in cultures and pallisades. Giemsa stain. 900 X.

and gram plus minus. Spores are not formed. Capsules have been demonstrated.

Cultural characteristics. On blood agar slants containing 8 per cent defibrinated horse blood, at the end of twenty-four hours, the growth was abundant with little tendency to spread, being viscid, grayish-white in appearance and producing hemolysis.

Nutrient agar containing 8 per cent horse serum gave growth equal to that on blood agar.

Loeffler's coagulated serum slants. After twenty-four hours small pin point areas of liquefaction were seen; these increased in size during continued incubation. At the end of fifteen days a large portion of the slant was liquefied.

Gelatin. After seven days at 22 C., liquefaction took place, although the growth was scant.

Potato. Little or no growth after four days.

Nutrient broth. Growth scanty and slow.

Nutrient broth with 8 per cent inactivated horse serum added. Good growth after four days at 36 C.

Nutrient broth with yeast extract added. Growth scant and slow.

Bordet medium containing 20 per cent horse blood. Growth fair.

Litmus milk. After seven days incubation the medium became more alkaline. Three distinct zones were plainly visible, the upper third a deep blue, the middle zone lavender and the bottom zone a light lavender containing coagulated casein.

Special media consisting of ox serum ultrafiltrate and mouse embryonic brain tissue. Growth fair after several days.

Optimum temperature, 36 C. The organism was killed at 59–60 C. in four minutes. The organism was facultative aerobic.

Biochemical characteristics. Indol was not produced. Dextrose, sucrose, maltose, xylose, mannitol, dulcitol, lactose and laevulose were inoculated. No acid or gas was produced, all of the inoculated media, after seven days, became more alkaline.

Serologic properties. Agglutination tests were made with sera from calves convalescent from the natural and experimental disease, with the following results:

Calf No.	Serum Dilutions 1:20	1:40	1:80	1:160	1:320	1:640
270 (control)	—	—	—	—	—	—
139	++++	++++	+++	++	+	—
140	+++	++	+	—	—	—
141	++++	++++	+++	++	+	—
143	++	+	—	—	—	—
148	++	+	—	—	—	—

Note: No. 140 was a field case whereas the others were produced experimentally from cultures of the hemolytic bacillus. Live and formalized antigens gave equally good results.

The five animals included in the agglutination tests were reinoculated with the culture of the hemolytic diplobacillus

along with control calf No. 279. None of these five animals showed any evidence of the disease, but the control animal exhibited a typical case of the disease on the fifth day. The organism is non-pathogenic to small laboratory animals except for white mice which may die after intra-abdominal injection of a young culture. Filtrates of ten-day old broth-serum cultures, when injected into laboratory animals, were observed to be non-toxic.

Experimental reproduction of keratoconjunctivitis in cattle and other ruminants. Pure cultures of the hemolytic bacillus produced typical disease when instilled into the eye of cattle, sheep and goats. Freshly isolated cultures were planted on blood agar slants and incubated for twenty-four hours at 36 C. The growth was washed off with nutrient broth and a few drops of the suspension were instilled into the eyes of normal animals. No difficulty was encountered in producing the disease by this method. To date, 18 Hereford calves, 1 sheep and 2 goats have been inoculated and in each was developed a clinical case of keratoconjunctivitis; some cases were unilateral whereas others were bilateral. Our observations of several of these experimentally produced cases are reported in detail as follows:

Hereford calf No. 141 was inoculated November 14, 1944 in both eyes with a suspension of culture isolated November 6, 1944 from field case No. 140. Nov. 15.—Slight lacrimation, left eye. Nov. 16.—Slight swelling of lower left lid, marked lacrimation, slight photophobia, clear mucous nasal discharge in long strands, slight opacity of the left cornea. Nov. 17.—Bilateral infection, marked photophobia, marked lacrimation, swelling of lower lids. Bilateral nasal discharge, slightly opacity of cornea. Nov. 18.—Marked bilateral corneal opacity, vision disturbed, marked lacrimation, both lower lids considerably swollen, conjunctivitis marked, blood vessels of the eye ball injected. Nov. 19.—Total bilateral corneal opacity, blind in both eyes, extensive lacrimation, marked swelling of lower lids, evidence of pain, much nasal discharge, (Fig. 5). Nov. 25.—Blind, total bilateral corneal opacity, much lacrimation, lower lids swollen, marked conjunctivitis, much nasal discharge. Nov. 29.—Condition not improved. Dec. 4.—Blind in both eyes, lacrimation decreased, swelling of lower lids subsiding, little nasal discharge. Dec. 11.—Corneal opacity clearing, ulcer on the cornea of right eye 6 mm. in diameter, vision less impaired, swellings of eye lids subsided. Started treatment, instillation of 1.5 per cent aqueous

Fig. 5. Experimental keratoconjunctivitis in calf No. 141, produced by installation of the culture. Corneal opacity, lacrimation, conjunctivitis.

solution of silver nitrate into both eyes daily. This calf made a complete recovery and was released December 29, 1944.

January 26, 1945: This calf, No. 141, was reinoculated with culture from calf No. 143, but any evidence of the disease failed to develop.

Hereford calf No. 145 was inoculated in both eyes November 24, 1944 with a suspension of culture isolated November 21 from case No. 141. Nov. 26.—Left eye, slight lacrimation with swelling of lower lid. Nov. 27.—Left eye—partial corneal opacity, lacrimation, photophobia, conjunctivitis, lower lid swollen, blood vessels of eye injected, nasal discharge. Right eye, slight lacrimation, some photophobia, lower lid swollen, slight conjunctivitis. Nov. 28.—Bilateral corneal opacity, vision disturbed, photophobia, lacrimation, swelling of lower lids, nasal discharge. Nov. 29.—Blind, marked corneal opacity, conjunctivitis, lacrimation, lower lids swollen, nasal discharge marked. Dec. 1.—Blind, corneal opacity complete, other phenomena same as on Nov. 29. Started treatment 1.5 per cent aqueous solution of silver nitrate instilled into eyes daily. No change was noted until December 18. Dec. 18.—Corneal opacity clearing, lacrimation decreasing, swelling of lids subsiding. Nasal discharge slight. Dec. 21.—Right eye—vision fair. Left eye—blind, but cornea clearing, photophobia decreasing, no nasal discharge, slight swelling of lower lid of left

eye only. Dec. 27.—Vision about normal, slight bilateral corneal opacity, no lacrimation, no photophobia, no nasal discharge. Dec. 30.—This animal still had a very small bilateral area of opacity, vision apparently normal. Animal was released.

Hereford calf No. 148 inoculated in both eyes January 6, 1945 with a suspension of a culture isolated from calf No. 143 on January 4, 1945 (Fig. 6).

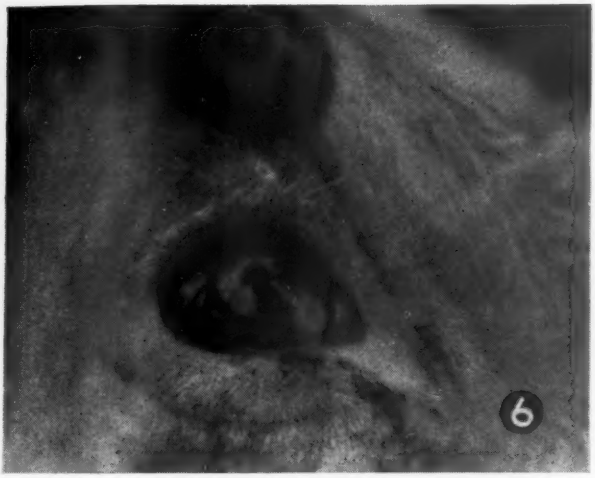

Fig. 6. Experimental disease in calf No. 148, produced by the diplobacillus in culture. Left eye—swelling of eyelids, lacrimation, conjunctivitis, opacity and ulceration of the cornea, loss of vision.

Jan. 10.—Bilateral swelling of lower lids, some photophobia, slight lacrimation. Jan. 11.—Slight bilateral corneal opacity, photophobia slight, marked lacrimation, conjunctivitis, ropy mucoid nasal discharge. Jan. 12.—Blind in both eyes, marked swelling of lower lid, lacrimation increased, blood vessels of eyes engorged. Jan. 15.—Corneal opacity of right eye clearing, marked lacrimation from left eye with swelling of the lower lid. Nasal discharge from both nostrils. Jan. 18.—Right eye normal. Left eye—complete opacity with loss of vision, marked lacrimation, photophobia, and nasal discharge. Jan. 28.—Right eye normal. Left eye blind, little lacrimation, some photophobia and nasal discharge. Jan. 29.—Right eye—lower lid swollen, slight lacrimation and photophobia. Left eye—corneal opacity clearing. Jan. 30.—Slight corneal opacity of both eyes, little photo-

phobia, slight swelling of lower lid of right eye. This calf made a complete recovery without treatment and was released February 8, 1945.

Sheep No. 20 was inoculated in both eyes January 24, 1945 with a suspension of culture isolated January 4, 1945 from experimental keratoconjunctivitis (calf No. 143).

Jan. 30.—Right eye small corneal area of opacity, slight photophobia, slight lacrimation, little swelling of lower lid. Conjunctivitis mild. Jan. 31.—Same as on Jan. 30. Vascular congestion of cornea. Feb. 1.—Right eye—small circumscribed corneal area of opacity (6 mm. in diameter). Some photophobia, little lacrimation, conjunctivitis and vascular congestion subsiding. Feb. 3.—Area of opacity slightly decreased, slight photophobia, very little lacrimation, no conjunctivitis. Feb. 7.—Very small area of opacity (2 mm.) of right eye, otherwise normal (Fig. 7). Feb. 10.—Right

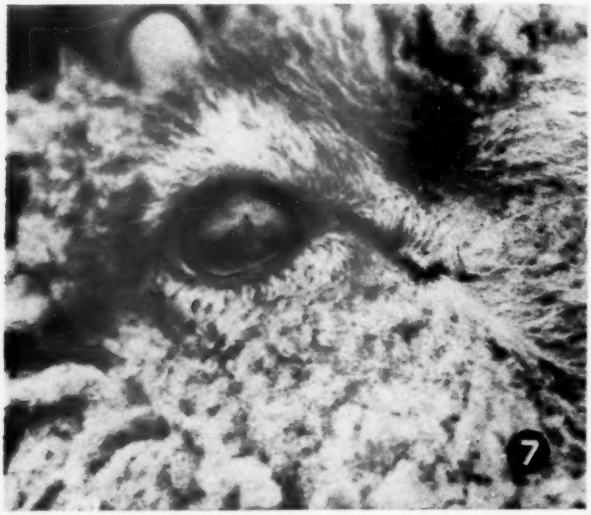

Fig. 7. Experimental disease in sheep No. 20, produced by a blood agar culture of the diplobacillus. Right eye—conjunctivitis, lacrimation, circumscribed shall area of corneal capacity.

eye—corneal area of opacity small, slight lacrimation, no photophobia. This sheep made a complete recovery and was released February 13, 1945.

Prevention and control. It can be seen from the foregoing observations and experimental results that keratoconjunctivitis in cattle is of bacterial origin, the hemolytic diplobacillus being

the specific agent. Since the disease is a systemic infection which confers immunity, its prevention by a specific bacterial vaccine is considered as a practicable continuation of this work.

At the present time no efficient method of control and treatment of the disease can be outlined other than strict segregation of the healthy from the infected animals and protection from sunlight and dust. Instillation of non-irritating chemicals, such as 1.5 per cent aqueous solution of silver nitrate, has given satisfactory results. Under range conditions, when the disease makes its appearance, it has been found advisable not only to separate the infected from the non-infected animals, but to treat all the animals once and the infected cattle twice or three times a week with silver nitrate.

Summary and Conclusions

Infectious keratoconjunctivitis of cattle on the Gulf Coast of Texas was investigated from epizootic and etiologic considerations. The disease is highly contagious among cattle, the eye and nasal discharges being the sources of infection. Although it is prevalent throughout the year, the highest incidence coincides with the summer season.

Microscopic studies of the conjunctiva of affected cattle revealed intra- and extracellular minute bodies which bore morphologic resemblance to rickettsiae, but no evidence could be produced as to their relationship to this disease. The disease can be readily transmitted experimentally in calves by contact with the eye or nasal exudates, but bacteria-free filtrates from the discharges are non-virulent. In few instances bacteria-free conjunctival scrapings from acute cases were obtained and inoculated into developing eggs. As a result, hemorrhagic lesions and deaths were noticed in certain proportions of incubated chick embryos, and on frequent occasions, microscopic examination of yolk sacs revealed coccoid bodies resembling rickettsiae or "inclusion bodies." However, attempts to infect the eye of susceptible calves with this bacteriologically sterile material invariably yielded negative results.

Systematic investigation of the bacterial flora of infectious keratoconjunctivitis revealed, in addition to various coccal organisms cultivated on ordinary media, a hemophilic and hemolytic bacillus which requires blood or serum for growth. Experimental keratoconjunctivitis in cattle, sheep and goats was produced by young cultures of this bacillus. In addition, agglutinins specific for this organism were found in the sera of cattle convalescent from the natural or experimental infection.

These results entitle us to the conclusion that the cultivated bacillus is the specific agent of infectious keratoconjunctivitis in cattle. On the basis of morphology, as well as cultural and pathogenic characteristics, it seems closely related if not identical with the Diplobacillus of Jones and Little, 1923; synonyme *Hemophilus bovis* (Hauduroy et al., 1937). Should the same organism be found in spontaneous keratitis of sheep and goats which proved susceptible to the experimental infection, then the designation *H. ruminantium* would seem more appropriate.

Acknowledgments

The authors wish to express their appreciation for the interest and coöperation in this work shown by Dr. H. Schmidt, Chief Division of Veterinary Science, Texas Agricultural Experimental Station, College Station, Texas.

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FIELD INVESTIGATIONS PERTINENT TO BULLIS FEVER

The Lone Star Tick, *Amblyomma americanum* (Linnaeus, 1758). Notes and observations from Camp Bullis, Texas[1]

JAMES M. BRENNAN[2]

Medical importance.

Amblyomma americanum, long recognized as a pest, exhibiting vicious biting propensities, is now acquiring more importance in medical entomology because of its recently proved association with disease transmission. All stages of the tick voraciously attack man as well as numerous species of mammals and birds. That this tick is exceptionally annoying and fully capable of demoralizing troops will be attested by anyone who has frequented woodlands where it is abundant.

Possessing unusually long, piercing mouth parts (the hypostome with its rows of retrorse "teeth"), *A. americanum*, especially the adult, can inflict severe and sometimes painful injury. When firmly attached, its removal may be attended with some difficulty if one does not wish to leave the mouth parts imbedded in the flesh to encourage secondary infection. In several cases among troops, scars (some of them still unhealed lesions) were observed which had persisted for as long as two years following the removal of this tick. In the author's personal experience, more than six months were required to effect the complete healing of a lesion after having removed an adult female which had been attached undetected for approximately two days.

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[1]This work was done with the approval of Brig. Gen. C. K. Nulsen, Commanding, Fort Sam Houston, Texas, and under direction of the Post Surgeon, Col. R. H. Duenner, through whom the necessary assistance and facilities were obtained. Received for publication March 30, 1945.

The writer acknowledges the kind services of Maj. H. L. Edgar, Inf., Executive Officer, Camp Bullis, Texas, in providing quarters and field laboratory for this project.

[2]Assistant Entomologist, Rocky Mountain Laboratory, Division of Infectious Diseases, National Institute of Health. Formerly Post Entomologist, Fort Sam Houston, Texas.

In the laboratory, Maver (1911) was able to transmit spotted fever in guinea pigs with *A. americanum* as an experimental vector. Anigstein (1942) reported four cases of spotted fever in Texas wherein *A. americanum* was strongly suspected to be the vector. Parker, Kohls and Steinhaus (1943) recovered the spotted fever rickettsia from *A. americanum* collected in Oklahoma. Both from circumstantial evidence and a demonstration of infected ticks in nature, this species is definitely established as a transmitter of spotted fever to man.

The infectious agent of American Q fever has been recovered repeatedly from *A. americanum* in Texas (Parker and Kohls, 1943; also Parker and Steinhaus, 1943).

According to Parker *et al.* (1937), there is a likelihood that *A. americanum* may be a potential vector of tularemia. Byfield *et al.* (1945) have provided circumstantial evidence associating this species with the occurrence of tularemia in troops in Tennessee.

Most recently a new disease entity designated as "Bullis fever" was described by Woodland, McDowell and Richards (1943). It is quite likely that this disease, occurring among troops at Camp Bullis, Texas, is rickettsial and that *A. americanum* is the vector (Livesay and Pollard, 1943; Anigstein and Bader, 1943; Bader and Anigstein, 1944).

This last named disease has been responsible for an extended series of investigations of which the data and observations recorded herein constitute but a small part.

The Tick.

Amblyomma americanum (Fig. 1) is a relatively small tick. The female has a single conspicuous white spot on the center of the back, from which is derived the common name "lone star tick." The smaller male has a few irregular white markings near the margins of the back. Nymphs and larvae are unmarked. All stages possess exceptionally long mouth parts, and for ticks, crawl with amazing speed. Cooley and Kohls (1944) give a complete technical description and other data.

The species is a "three-host" tick, the engorged larvae and nymphs dropping from their host animal for molting. Mating

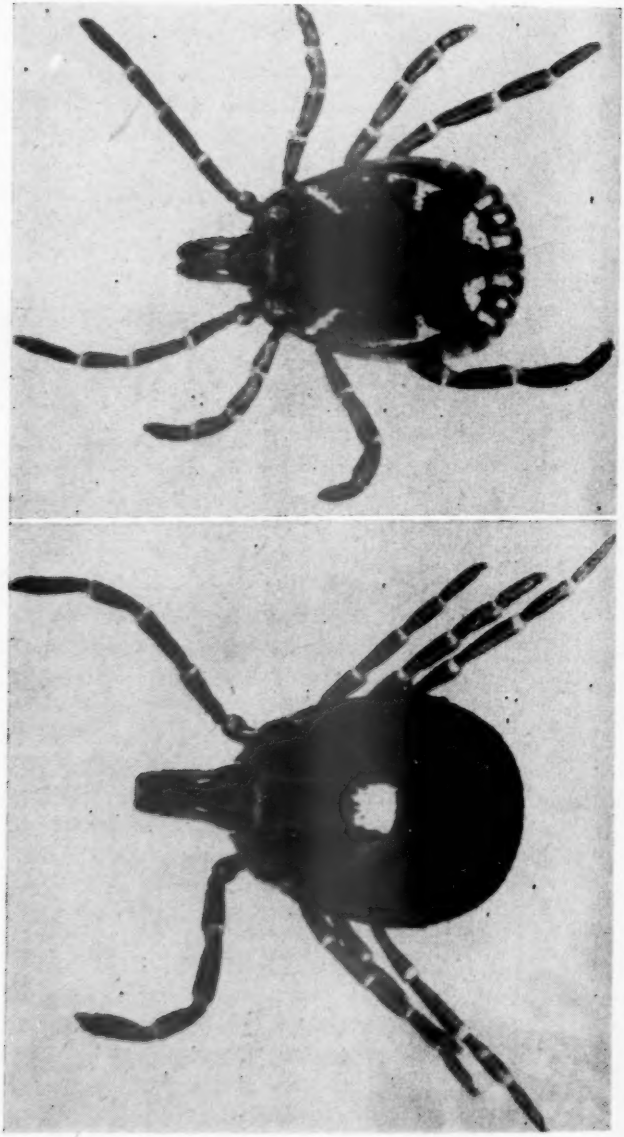

Fig. 1. *Amblyomma americanum.* Left, female. Right, male.

takes place on the host, from which the fully engorged female drops to the ground to lay her several thousand eggs and die. All stages are frequently present on the same host. The adults show a preference for large mammals. Unfed adults, nymphs and larvae have been observed to live 13, 16 and 9 months respectively (Hooker, Bishopp and Wood, 1912).

Survey.

Coincident with a faunal survey of Camp Bullis (Brennan, 1945), and with the collecting of more than 50,000 *A. americanum* for laboratory use in Bullis fever studies, an excellent opportunity was afforded for obtaining data on tick-host relationships, as well as certain other miscellaneous information.

The tabulated host data were secured from June 8 to October 1, 1943. Some additional studies were made irregularly until the end of the year, and then from February to August 1944.

A brief description of the Camp Bullis area was given in an earlier report by Brennan *(op. cit.)*. It will suffice to repeat here that the military reservation comprises about 30,500 acres and it is also a federal game preserve. Faunal, climatic, and topographical features afford an ideal tick habitat.

Early in the work a survey of the entire area demonstrated *Amblyomma americanum* to be unquestionably the most abundant tick of the region. Its numbers constitute an estimated 95 per cent or more of the total tick population. This information was derived by random sampling of ticks from the ground and vegetation supplemented by host data.

Appraising the tick population on the ground was attempted by establishing 71 stations chosen at random from a map. They were spaced at fairly equal intervals throughout the reservation. As each station was visited, two 1/10 acre plots were laid out within a few hundred yards of each other. No deviation was permitted for topographical features. Each plot at each station was systematically and thoroughly dragged, using a modification of the conventional white canton flannel flag (Kohls, 1937), i.e., with the staff cut to conform to the width of the cloth which is dragged over the ground behind the operator by means of a convenient length of rope.

Left, female. Right, male.

Tick counts were recorded for adults and nymphs. The maximum number of these stages in aggregate, per 1/10 acre plot, was 150. About 90 per cent of these were nymphs. The average number was 17.5. Almost invariably the number of nymphs greatly exceeded the number of adults. This sampling was done during the last half of June 1943. Since no practicable method presented itself for determining accurately the percentages of nymphal and adult populations taken by dragging, and in the light of additional experience, it is indicated that the figures belie the facts. Except that dragging never showed the presence of any other species but *A. americanum* and served merely as an index of distribution, the data obtained are not believed to be representative, or of statistical value.

Later experience demonstrated the relative ineffectiveness of dragging both as a means for collecting and for sampling, because the ticks normally do not occur on vegetation. Boardman (1944) made similar observations on ticks, species not mentioned, in the southern states. At no time throughout the summer of 1943 were adults and nymphs of *A. americanum* recorded from vegetation. These stages were almost invariably on the ground, sometimes several inches beneath the overlying litter and duff, occasionally in the crowns of grasses. Only the larvae occurred habitually in clusters on grass blades, etc., a few inches above the soil surface. However, on April 27, 1944, the author and Lt. G. F. Augustson did manage to secure one record of adults above the surface of the ground. They were observed in considerable numbers, some nearly waist-high on sedges in association with trees along a dry stream bed. The day was cool and cloudy. The following day was much warmer and clear. A return trip to the same location showed just an occasional tick on the vegetation.

In spite of the all too frequent, but unverified, reports from bivouacking troops that the ticks drop on them from the trees (on one occasion an officer insisted that a tick "flew" in his eye), the writer believes that it is a rare phenomenon to find unfed adults and nymphs in this climate anywhere except on the ground. Conceivably, such behavior is influenced by the characteristic hot, dry summers. The ticks retire for protection

beneath litter, and most certainly benefit by the retained moisture there. Circumstantial evidence indicates that *A. americanum* will crawl for short distances in search of a favorable environment.

Abundance and distribution.

Although neither time nor facilities permitted computing the actual number of ticks per any given unit, certain observations supplemented by collecting records indicated an abnormally large *A. americanum* population. The writer could find no records in literature, through correspondence, or from verbal information, of a greater concentration of this species elsewhere in the United States. Undoubtedly various ecological factors induce this excessive tick population, but it is presumed that the primary predisposing cause originates in the prodigious numbers of mammals and birds. Scouting areas adjacent to and within a ten-mile zone around Camp Bullis, showed *A. americanum* to be almost negligible as compared to its prevalence on the reservation, with the exception that the ticks were found to be fairly abundant on one small estate where several deer were confined.

A credible report from an Army medical officer stated that 294 ticks were detached from an enlisted man on one occasion. Questioning revealed that the ticks were adults and nymphs, the former more numerous. The exposure time was not known.

The maximum number of adults removed from the head of a deer, *Odocoileus virginianus*, was 910. The greatest number of nymphs from the head of another specimen was 1160. As a rule, larvae on deer were much too numerous to warrant the feasibility of counting. From a young gray fox, *Urocyon cinereoargenteus*, a total of 3064 ticks were recorded. Of this number, 21 were adults, more than 2000 were larvae, and the remainder were nymphs. From a jack rabbit, *Lepus californicus merriami*, 1183 ticks were recovered. About two-thirds were larvae, one-third nymphs, and only two were adults. From a bobwhite quail, *Colinus virginianus*, 1952 larvae and a few nymphs were removed.

Ground collecting complemented the above cases. On July 24, 1943, four men, working as rapidly as possible and without moving from the spot, collected 4086 adults beneath a small juniper tree. (Fig. 2.) A re-check under this tree one year later yielded only about 300 adults. At another similar location, the writer collected nearly 1200 adults in less than three hours. In both places the trees were relatively isolated from others of their kind and were surrounded by undergrowth except on one exposed side. The litter, composed principally of needles, was four to six inches deep. There were no signs, recent or old, of animal beds. Other areas of like nature were frequently highly productive.

(Photograph of site of collecting 4086 adults from ground)

Fig. 2. Typical *Amblyomma americanum* habitat. Beneath this juniper, 4086 adults were collected.

An effort was made to determine whether any part of Camp Bullis was at variance with any other part relative to tick density. The distribution and abundance of *A. americanum* exhibited no well defined pattern. The species was focalized wherever favorable ecological factors existed.

The tick was not present in semi-barren areas or grasslands. Roadsides were tick-free. Ticks were frequently encountered along the more obscure trials, however. For reasons suggested before, i.e., protection and need for moisture, the ticks were limited to thicket and woodland. Heaviest concentrations occurred in areas dominated by juniper and scrub oak, whether dense or sparse. There was no apparent difference in abundance in the vicinity of animal beds or in places showing no animal sign. For the most part, both nymphs and adults could be collected from the ground in greater numbers and more rapidly in the immediate vicinity of junipers and oaks where the litter was fairly deep.

Seasonal trends.

On June 8, 1943, both unfed and feeding adults and larvae of *A. americanum* were plentiful. Unfed nymphs were scarce and feeding nymphs moderately abundant. By the end of the month there had been no consequential difference in the adult and nymphal populations, but there was considerable reduction in the number of larvae. By the end of July, the adults were observed in diminishing numbers, tapering off noticeably in mid-August, concurrent with a gradual rise for both nymphs and larvae. By the end of August, the nymphs and larvae were rapidly approaching a peak. At this time, the larvae were much more numerous than in early June. The end of September brought about a general decline in all stages of unfed ticks, but feeding nymphs and larvae were still quite numerous.

In an entire day afield on October 14, only one unfed adult male was found. Again on November 4, one unfed adult female was collected. On this latter date, two nymphs had attached to the writer.

The first reports of adult tick activity for 1944 were received early in February. Complaints were registered by troops on bivouac. An investigation confirmed mild tick activity, but the low incidence of tick bite did not justify the nature of the reports.

Later in February and in March, with the coöperation of three battalions, the following information was obtained:

In the first group, having a mean strength of approximately 850, which had been on bivouac for the week ending February 21, only 83 men experienced tick bite. No one reported more than three ticks attached for the whole period, and 69 men reported only one tick.

In the second group, on bivouac from February 19 to 27, records were obtained from 500 men. Tick bite was reported by 287, or about 57 per cent. The maximum number of ticks attached to one man one day was 35. A few men reported more than 20, but the great majority had less than 5 tick bites for the whole period.

Information from the third group corresponded closely to that from the second group. This battalion of 757 officers and men were on bivouac from February 27 to March 4. Tick bite was reported by 427, or nearly 57 per cent. The greatest number of ticks attached to one man for one day was 14.

Having been given preliminary instruction, most of the men made an effort to distinguish between adults and nymphs, however, others reported indiscriminately. It was derived, after considerable questioning, that for the most part, tick bites were due to adults, and in a few instances to nymphs. Also, subsequent observations in the bivouac areas showed a preponderance of adults.

The adult population increased gradually until attaining a peak in late May, then following a course similar to that observed in 1943. The nymphal population increased more rapidly, apparently reaching a peak about mid-May at which time unfed nymphs outnumbered the unfed adults by a greater than 3 to 1 ratio. The first record on unfed larvae was obtained on June 9, at which date in 1943, larvae were quite abundant. The larvae attained a peak in late June, more or less leveling off through July, then dropping noticeably by mid-August. Nymphs were still quite abundant, no marked reduction in their numbers was noticed throughout the summer.

Summarizing the seasonal trends in the tick population for 1943 and 1944: Adults reached a peak in late May or early June, leveled off until the end of July, then decreased considerably by mid-August. There was evidence of one nymphal peak in 1943 occurring late in August, but in 1944 a peak was attained in mid-May with no marked reduction in numbers up to mid-August, when observations were discontinued. Two larval peaks were recorded for 1943 in early June and late August. One larval peak was recorded for 1944 in late June. There was little or no tick activity from October through January. Adults and nymphs became active in February, larvae in late spring.

In the light of present information, no plausible explanation can be offered for anomalies in the seasonal pattern. The need for additional and more comprehensive investigation is recognized.

Collecting methods.

The need for large numbers of living ticks, both fed and unfed, has been indicated. With the major portion of the disease transmission studies being conducted at the Rocky Mountain Laboratory, Hamilton, Montana, it devolved upon the writer not only to make provision for ample quantities of ticks, but also to devise means for shipping in order to insure the least possible mortality. To this end, many suggestions were accepted and tried until a better method presented itself. Ideally, a scheme for collecting, packing and shipping, which would require the least amount of time, effort and handling of ticks was highly desirable.

Obtaining fed ticks necessitated the collecting of many mammals and birds. This was accomplished by both shooting and trapping. Carcasses were sacked in muslin bags, or in their absence, double or triple sacked in brown paper bags. The bags were tied securely. In the case of deer, only the heads were taken, since it had been discovered previously that more than 90 per cent of the engorging ticks were attached there. It was desirable to hold specimens at room temperature for several hours before collecting the ticks in order that most of

them would be detached. When host material accumulated more rapidly than could be examined, refrigeration of sacked specimens at about 50° F. was essential.

Preparatory to transferring ticks to collecting tubes, a carcass was placed in a large, shallow, white porcelain pan, preferably on a sheet of white canton flannel. The sack was turned inside out or torn open. It was necessary to pluck all birds. The ticks were handled with forceps.

In the collecting of unfed ticks from the ground the customary dragging technique was attempted. The ineffectiveness of this procedure has been mentioned. It was soon discovered that by scouting for areas of heavy concentration, collecting could be accomplished efficiently. The scouting was continued for the entire collecting season, for it was desired to secure ticks from as many different localities as possible throughout the reservation.

When a general region for the day's collecting had been decided, collectors were instructed to work separately, walking slowly from one place to another, pausing for a half-minute or so when a seemingly favorable tick habitat was noticed. The presence or absence of ticks was readily detected. Some additional observation was necessary to estimate whether ticks were present in sufficient numbers to make collecting feasible; if so, all collectors would assemble. By seating themselves amidst the activated ticks, collecting involved merely the removal of specimens from the clothing with forceps and transferring them to containers. When ticks were not coming fast, the collector would place a square of white flannel on the ground nearby and examine it from time to time in addition to his clothing. If the collecting cloth were saturated with old perspiration, it appeared to produce more effective results.

The above procedure was found to be quite satisfactory, so that no modifications were required. However, it was necessary to alter certain other steps in the method of handling.

Ticks were first collected in homeopathic or shell vials. After several were inside, and since *A. americanum* crawls rapidly, frequent tapping of the vial was necessary to knock the specimens down before lifting thumb or cork to introduce another.

At best, some occasionally escaped. Filled vials were held at 50° F. until the ticks were inactivated. On removal from refrigeration, it was necessary to work quickly, tapping the vials so that the inactive ticks would "ball-up." They were then transferred *en masse* to pill boxes, each of which was sealed with adhesive tape. A box two inches in diameter by three-fourths inch deep would accommodate 300 to 400 adults. The pill boxes were packed in a mailing tube with a moist cotton pad at the bottom and top to provide humidity. Shipment was made by air mail.

Although effectual, the technique just described is not efficient, and is not recommended. It involved too much handling of ticks, subsequent loss of time, and refrigeration was required. Sometimes the moisture within a mailing tube was sufficient to cause a pill box to break open in transit.

Adopting the use of culture tubes, of any convenient size, prepared as described below, eliminated much of the above procedure. A tube five inches long by one-half inch in diameter would accommodate about 200 adults. Before collecting, a very tight, non-absorbent cotton plug was forced to the surface of approximately an inch of water in the tube. In the field a cork was used to retain the ticks while collecting. After filling such a "wet tube" the cork was immediately replaced by a cotton stopper. The lot was then ready for shipment.

But there were disadvantages to be overcome. There still existed the problem of occasionally losing ticks when introducing another specimen into the tube, and before a day's collecting was over, the constant tube-tapping became quite irksome.

The cork was replaced by a small sheet of good quality rubber stretched lightly over the mouth of the tube and secured by rubber bands. A tiny slit was made in the center of the sheet for receiving ticks from forceps. The rubber was obtained from discarded surgical gloves.

The use of this device facilitated the rapid collecting of ticks, but a disturbing problem was created. The rubber sheet inhibited ventilation, causing condensation in the tubes.

This defect was corrected by discarding the use of culture tubes and substituting glass tubing cut to desired lengths. Such a tube was prepared for collecting by forcing a tight non-absorbent cotton plug to within an inch or two of one end. The rubber sheet was attached to the other end (Fig. 3a). Thus sufficient ventilation was furnished while collecting. After the sheet was replaced by a cotton stopper, water was added to the opposite end of the tube and then a cork was inserted (Fig. 3b). Dipping this end of the tube in paraffin would insure sealing. Although not tried, the essential humidity might well be provided by substituting wet sand or sawdust for the water. In preparing these tubes, emphasis must be placed on the compactness of the non-absorbent cotton plug between the water and the ticks. To prevent seepage, the plug must fit so tightly that the greatest amount of pressure that the glass will withstand should be exerted in forcing the cotton in place.

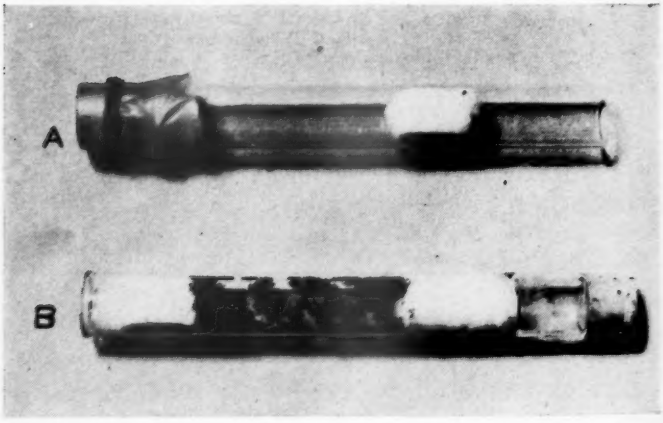

Fig. 3. a. Glass tube prepared for collecting ticks.
b. The same, ready for shipment.

Host data.

The accompanying tables include only data obtained from June 8 to October 1, 1943. They demonstrate the wide range

of wild hosts for *A. americanum* and are designed for comparing the relative incidence and abundance of all its stages by host species. Tables I and II, pertaining to mammals, should be used together, likewise for tables III and IV, relative to birds.

Amblyomma americanum was not present on the following species of birds. The figure preceding the name indicates the number of specimens examined:

3 *Coragyps atratus* (Black vulture)
6 *Pyrocephalus rubinus* (Vermilion flycatcher)
6 *Dryobates scalaris* (Ladderback woodpecker)
5 *Penthestes carolinensis* (Plumbeous chickadee)
5 *Petrochelidon albifrons* (Cliff swallow)
1 *Icterus spurius* (Orchard oriole)
3 *Myiarchus cinerascens* (Ash-throated flycatcher)
1 *Passer domesticus* (English sparrow)
1 *Querquedula discors* (Blue-winged teal)
1 *Tringa sollitaria* (Solitary sandpiper)
2 *Molothrus ater* (Cowbird)
1 *Falco sparverius* (Sparrow hawk)
2 *Troglodytidae* (Wren)
1 *Tyrannidae* (Flycatcher)

From table I, it will be noted that the tick was recorded from all species of mammals examined. Both armadillos from which one nymph each was removed were immature.

In the spring and summer of 1944, sixteen white-footed mice representing two species *(Peromyscus leucopus texanus* and *P. pectoralis laceianus)* were trapped and examined. *A. americanum* was not present on any specimen. A few more specimens of the ground squirrel *(Citellus mexicanus parvidens)* were taken. These also were uninfested.

From these observations and the tabular data, as well as because of the relative scarcity of the smaller rodents, it is concluded that they are not a significant factor in the maintenance of the *A. americanum* population at Camp Bullis. The armadillo, although very abundant, is likewise of no importance. Presumably due to morphological or physiological reasons, or both, it is not a suitable host.

TABLE I

Incidence of adults, nymphs and larvae of Amblyomma americanum on mammals at Camp Bullis, Texas

Mammalian Species	No. specimens examined					No. specimens infested					Percent infested with adults					Percent infested with nymphs					Percent infested with larvae				
	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period
Odocoileus virginianus subsp. (White-tailed deer)	5	8	7	24	44	5	8	7	24	44	100	100	100	71	84.1	100	100	100	100	100	100	100	100	100	100
Lepus californicus merriami (Jack rabbit)	11	8	19	11	49	10	8	9	6	33	27.3	62.5	5.3	9.1	20.4	90.9	100	47.4	54.5	67.3	72.7	50	15.7	9.1	32.7
Sylvilagus floridanus chapmani (Cottontail rabbit)	1	4	1	3	9	1	3	1	3	8	0	25	0	0	11.1	100	75	100	100	88.8	0	50	100	66.7	55.5
Urocyon cinereoargenteus subsp. (Gray fox)	2	1		2	5	2	1		2	5	100	100		0	60	100	100		100	100	100	100		100	100
Procyon lotor fuscipes (Raccoon)	3	1	5	2	11	3	1	5	2	11	33	0	20	50	27.2	100	100	100	100	100	100	100	0	100	54.5
Mephitis mephitis mesomelas (Striped skunk)			5	3	8			5	3	8			0	0	0			100	100	100			80	66.7	75
Conepatus mesoleucus mearnsi (Hog-nosed skunk)			2	1	3			2	1	3			50	0	33			100	100	100			50	0	33
Sciurus niger limitis (Fox squirrel)	1		10		11	1		6		7	0		0		0	100		60		63.6	100		60		63.6
Didelphis virginianus (Opossum)		1	5		6		0	5		5		0	0		0		0	20		16.7		0	100		86.7
Bassariscus astutus flavus (Cacomistle)		1		1	2		1		1	2		100		0	50		100		100	100		0		0	0
Dasypus novemcinctus texanus (Armadillo)	7		14	4	25	1		1	0	2	0		0	0	0	14.3		7.2	0	8	0		0	0	0
Citellus mexicanus parvidens (Rio Grande ground squirrel)			1		1			1		1			0		0			0		0			100		100
Citellus variegatus buckleyi (Rock squirrel)		1			1		1			1		0			0		100			100		100			100
Rattus rattus alexandrinus (Roof rat)				1	1				1	1				0	0				0	0				100	100
Sigmodon hispidus texianus (Cotton rat)			2		2			1		1			0		0			50		50			50		50

TABLE II

Abundance of adults, nymphs and larvae of Amblyomma americanum on mammals at Camp Bullis, Texas

Mammalian Species	Average No. adults					Average No. nymphs					Average No. larvae				
	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period
Odocoileus virginianus subsp. (White-tailed deer)	1.2	1.0	1.0	0.3	0.6	1.2	1.0	1.0	0.3	0.6	1.2	1.0	1.0	0.3	0.6
Lepus californicus merriami (Jack rabbit)	0.3	0.6	0.1	0.1	0.2	3.3	3.0	1.1	1.0	1.8	3.3	3.0	1.1	1.0	1.8
Sylvilagus floridanus chapmani (Cottontail rabbit)	0	0.5	0	0	0.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Urocyon cinereoargenteus subsp. (Gray fox)	1.0	1.0		0	0.6	1.0	1.0		1.0	1.0	1.0	1.0		1.0	1.0
Procyon lotor fuscipes (Raccoon)	0.3	0	0.2	0.5	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mephitis mephitis mesomelas (Striped skunk)			0	0	0			1.0	1.0	1.0			1.0	1.0	1.0
Conepatus mesoleucus mearnsi (Hog-nosed skunk)			0.5	0	0.3			1.0	1.0	1.0			1.0	1.0	1.0
Sciurus niger limitis (Fox squirrel)	0		0		0	0		0		0	0		0		0
Didelphis virginianus (Opossum)		0	0		0		0	0		0		0	0		0
Bassariscus astutus flavus (Cacomistle)		1.0		0	0.5		1.0		0	0.5		1.0		0	0.5
Dasypus novemcinctus texanus (Armadillo)	0		0	0	0	0		0	0	0	0		0	0	0
Citellus mexicanus parvidens (Rio Grande ground squirrel)			0		0			0		0			0		0
Citellus variegatus buckleyi (Rock squirrel)		0			0		0			0		0			0
Rattus rattus alexandrinus (Roof rat)				0	0				0	0				0	0
Sigmodon hispidus texianus (Cotton rat)			0		0			0		0			0		0

Abundance of adults, nymphs and larvae of Amblyomma americanum on mammals at Camp Bullis, Texas

Mammalian Species	Max. No. adults per mammal				Max. No. nymphs per mammal				Max. No. larvae per mammal				Average No. adults per mammal					Average No. nymphs per mammal					Average No. larvae per mammal				
	June	July	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period
Odocoileus virginianus subsp. (White-tailed deer)	910	248	140	61	150	443	1160	1150	*	*	*	*	287	103.9	50.6	64	62.8	49.2	212.6	464.1	415.6	347.1	*	*	*	*	*
Lepus californicus merriami (Jack rabbit)	7	3	1	1	450	81	50	14	*	*	*	*	0.9	1.1	0.05	0.09	0.4	71.4	24.3	5.4	2.9	22.7	*	*	*	*	*
Sylvilagus floridanus Chapmani (Cottontail rabbit)	0	1	0	0	78	29	27	32	0	258	100	2	0	0.25	0	0	0.1	78	9.2	27	17.6	21.7	0	66.5	100	0.6	40.9
Urocyon cinereoargenteus subsp. (Gray fox)	21	13		0	55	100		115	*	*		*	19.5	13		0	10.4	35	110		97.5	75	*	*		*	*
Procyon lotor fuscipes (Raccoon)	1	0	1	1	16	24	69	108	67	4	0	250	0.3	0	0.2	0.5	0.27	11	24	23.8	102.5	35.5	52	4	0	250	60
Mephitis mephitis mesomelas (Striped skunk)			0	0			36	11			*	*			0	0	0			15.4	9.7	13.3			*	*	*
Conepatus mesoleucus mearnsi (Hog-nosed skunk)			1	0			92	2			29	0			0.5	0	0.3			53	2	36			14.5	0	9.7
Sciurus niger limitis (Fox squirrel)	0		0		7		28		8		94		0		0		0	7		4.6		4.8	8		18		17
Didelphis virginianus (Opossum)		0	0			0	2			0	9			0	0		0		0	0.6		0.5		0	7.6		6.7
Bassariscus astutus flavus (Cacomistle)		3		0		3		10		0		0		3		0	1.5		3		10	6.5		0		0	0
Dasypus novemcinctus texanus (Armadillo)	0		0	0	1		1	0	0		0	0	0		0	0	0	0.14		0.07	0	0.08	0		0	0	0
Citellus mexicanus parvidens (Rio Grande ground squirrel)			0				0				2				0		0			0		0			2		2
Citellus variegatus buckleyi (Rock squirrel)		0				2				1				0			0		2			2	1				1
Rattus rattus Alexandrinus (Roof rat)				0				0				4				0	0				0	0				4	4
Sigmodon hispidus texianus (Cotton rat)			0				1				1				0		0			0.5		0.5			0.5		0.5

*Larvae so numerous that counting was impracticable.

TABLE III

Incidence of Amblyomma americanum nymphs and larvae on birds at Camp Bullis, Texas

Avian Species	Number of specimens examined					Number of specimens infested with ticks					Percent infested with nymphs					Percent infested with larvae				
	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period
Richmondena cardinalis (Cardinal)	4	12	30	17	63	4	8	27	16	55	25	66	66	88	68	100	17	90	76	73
Colinus virginianus (Bobwhite quail)	4	13	17	4	38	4	13*	17	4	38	25	92	82	100	82	100	77	94	100	89
Aimophila ruficeps (Rock sparrow)	1	3	12	4	20	1	2	11	4	18	0	67	42	100	45	100	0	67	50	55
Thryomanes bewicki (Bewick's wren)	1	1	10		12	0	1	8		9	0	0	60		50	0	100	80		75
Spizella pusilla (Field sparrow)	1	3	3	4	11	0	1*	2	4	7	0	0	33	75	36	0	0	33	75	36
Chondestes grammacus (Western lark sparrow)	7	7	11	5	30	1	3	4	2	10	0	0	18	20	10	14	43	27	20	27
Zenaidura macroura (Mourning dove)	4	3	12	25	44	0	1	2	6	9	0	33	0	24	16	0	0	17	16	14
Mimus polyglottos (Mockingbird)	4	7	4		15	2	1	1		4	0	14	0		7	50	0	25		20
Baeolophus atricristatus (Sennett's titmouse)	3	2	6	1	12	1	0	2	0	3	0	0	33	0	17	33	0	0	0	8
Piranga rubra (Summer tanager)	3	2	5	1	11	0	0	2	0	2	0	0	25	0	9	0	0	25	0	9
Meleagris gallopavo (Wild turkey)		1	1		2		1	1		2		100	100		100		100	100		100
Catherpes mexicanus (Canyon wren)	1		1		2	1		1		2	0		100		50	100		0		50
Phalaenoptilus nuttalli (Poor will)			5	3	8			4	1	5			40	33	37			60	33	50
Antrostomus carolinensis (Chuck-wills-widow)			1		1			1		1			0		0			100		100
Icteria virens (Yellow-breasted chat)	1	1			2	1	1			2	0	100			50	100	100	0	0	100
Spinus psaltria (Arkansas goldfinch)			2		2			2		2			50		50			100		100
Thryothorus ludovicianus				1	1				1	1				100	100				100	100

(Carolina wren)																						
Geococcyx californianus			1		1				1		1			100		100			100		100	
(Road runner)																						
Guiraca caerulea	1	1	1		3	0	0		1		1	0	0	0		0		0	0	100		33
(Western blue grosbeak)																						
Cathartes aura	1				1	1*					1	100				100		0				0
(Turkey vulture)																						
Muscivora forficata	1				1	1					1	0				0		100				100
(Scissor-tailed flycatcher																						
Myiarchus crinitus			1	1	2				1	0	1			100	0	50				0	0	0
(Crested flycatcher)																						
Columbigallina passerina				1	1					1	1				0	0					100	100
(Ground dove)																						
Myiochanes richardsonii				1	1					1	1				100	100					0	0
(Western wood pewee)																						
Passerina ciris	3	2	1		6	1	0		1		2	0	0	100		17		33	0	0		17
(Painted bunting)																						
Buteo borealis			1		1				1		1			100		100				100		100
(Red-tailed hawk)																						
Buteo swainsoni			1		1				1		1			100		100				100		100
(Swainson's hawk)																						
Carpodacus mexicanus			1	1	2				0	1	1			0	0	0				0	100	50
(House finch)																						
Coccyzus americanus		1	1		2		1		0		1		100	0		50			100	0		50
(Yellow-billed cuckoo)																						
Lanius ludovicianus		1	1		2		0		1		1		0	100		50			0	0		0
(White-rumped shrike)																						
Empidonax minimus		1	1	2	4		0		0	1	1		0	0	50	25			0	0	0	0
(Least flycatcher)																						
Dendroica caerulescens	1				1	1					1	0				0		100				100
(Black-throated blue warbler)																						
Fringillidae	3				3	2					2	33				33		67				67
(Sparrow)																						

*1 adult tick present.

TABLE IV

Abundance of Amblyomma americanum nymphs and larvae on birds at Camp Bullis, Texas

Avian Species	Maximum number of nymphs per bird				Maximum number of larvae per bird				Average number of nymphs per bird					Average number of larvae per bird				
	June	July	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.	Period	June	July	Aug.	Sept.	Period
Richmondena cardinalis (Cardinal)	2	7	3	5	7	6	52	5	0.5	1.3	1	2	1.3	4.3	0.6	5.1	2	3.4
Colinus virginianus (Bobwhite quail)	5	16	11	8	1952	700	78	11	1.3	6.3	3.9	6.8	4.7	489.5	65.6	28.9	9.3	87.8
Aimophila ruficeps (Rock sparrow)	0	1	2	7	2	0	6	2	0	0.7	0 6	3	1	2	0	1.6	0.8	1.2
Thryomanes bewicki (Bewick's wren)	0	0	2		0	8	33		0	0	1.1		0.9	0	8	9.8		8.8
Spizella pusilla (Field sparrow)	0	0	1	2	0	0	5	36	0	0	0.3	1	0.4	0	0	1.6	10	4
Chondestes grammacus (Western lark sparrow)	0	0	2	4	6	4	2	2	0	0	0.3	0.8	0.2	0.9	1.1	0.4	0.4	0.7
Zenaidura macroura (Mourning dove)	0	4	0	2	0	0	4	1	0	1.3	0	0.4	0.3	0	0	0.6	0.2	0 4
Mimus polyglottos (Mockingbird)	0	1	0		1	0	6		0	0.1	0		0.1	0.5	0	1.5		0.5
Baeolophus atricristatus (Sennett's titmouse)	0	0	1	0	5	0	0	0	0	0	0 3	0	0.2	1.7	0	0	0	0.4
Piranga rubra (Summer tanager)	0	0	1	0	0	0	10	0	0	0	0.3	0	0.1	0	0	2.5	0	0.9
Meleagris gallopavo (Wild turkey)		59	18			78	13			59	18		38.5		78	13		45.5
Catherpes mexicanus (Canyon wren)	0		2		4		0		0		2		1	4		0		2
Phalaenoptilus nuttalli (Poor will)			1	3			38	3			0.4	1	0.6			8	1	5.3
Antrostomus carolinensis (Chuck-wills-widow)			0				1				0		0			1		1
Icteria virens (Yellow-breasted chat)	0	7			9	30			0	7			3.5	9	30			19.5
Spinus psaltria (Arkansas goldfinch)			1				1				0.5		0.5			1		1

Thryothorus ludovicianus (Carolina wren)				3					2					3	3					2	2
Geococcyx californianus (Road runner)			3				25				3			3				25			25
Guiraca caerulea (Western blue grosbeak)	0	0	0		0	0	6		0	0	0	0	0	0	0	0	6				2
Cathartes aura (Turkey vulture)	4				0				4					4	0						0
Muscivora forficata (Scissor-tailed flycatcher	0				1				0					0	1						1
Myarchus crinitus (Crested flycatcher)			2	0			0	0			2	0	1				0	0	0		0
Columbigallina passerina (Ground dove)				0				1				0	0						1		1
Myiochanes richardsonii (Western wood pewee)				2				0				2	2						0		0
Passerina ciris (Painted bunting)	0	0	1		2	0	0		0	0	1		0.2		0.7	0	0				0.3
Buteo borealis (Red-tailed hawk)			12				4				12		12				4				4
Buteo swainsoni (Swainson's hawk)			3				2				3		3				2				2
Carpodacus mexicanus (House finch)			0	0			0	1			0	0	0				0		1		0 5
Coccyzus americanus (Yellow-billed cuckoo)		2	0			13	0			2	0		1			13	0				6.5
Lanius ludovicianus (White-rumped shrike)		0	1			0	0			0	1		0.5			0	0				0
Empidonax minimus (Least flycatcher)		0	0	1		0	0	0		0	0	0 5	0.3			0	0	0			0
Dendroica caerulescens Black-throated blue warbler)	0				1				0				0		1						1
Fringillidae (Sparrow)	10				40				3.3				3.3		18.3						18.3

It is immediately perceived that among the mammals the deer is the dominant host for adults, if not also for nymphs and larvae. As stated elsewhere, the great majority of ticks are attached to the head regions, primarily the ears. Specimens were observed with the ears actually drooping from the weight of the engorging ticks. In one exceptional case, an adult was detached from the eyeball of a fawn. The only instance where ticks were observed in numbers well distributed on the body of a deer was an animal dying from a severe screwworm infestation.

Despite the numerous ticks on deer, no animals were observed which appeared to suffer any ill effects from the infestation.

Six deer examined during the last half of October averaged 1 adult and 36 nymphal ticks. A moderate number of larvae were present on all. Only a few nymphs were found on a fox taken this same period.

Among the smaller animals, the ground dwelling and bush inhabiting birds are the most important hosts for the immature stages of *A. americanum*. Because of their abundance and habits, it may be presumed that the birds are of equal importance with the mammals in supporting and dispersing the tick population.

As with the mammals, none of the heavily infested birds appeared to suffer any ill effects from tick bite. The quail, a female with young, with nearly 2000 ticks attached was healthy and vigorous.

In conclusion, attention is directed to the interpretation of the tabular data. Proper evaluation is contingent upon a knowledge of animal behavior as well as of animal populations. For example, from table IV, under the larval average column, it appears that the larval index for the bobwhite quail is much higher than for the cardinal, however, the latter's abundance on the reservation was considerably greater, therefore it is conceivable that the cardinal as a host may rank equally with the quail. Other similar examples could be cited.

Summary

1. *Amblyomma americanum*, a consequential pest, is receiving increasing recognition in medical entomology as a disease vector. Most recently this tick has been associated with the transmission of a new rickettsial disease entity, Bullis fever.

2. *A. americanum* is exceptionally abundant at Camp Bullis, Texas, where it was shown to have a wide range of hosts. The unfed ticks were found to occur in focalized distribution throughout the military reservation.

3. Methods for collecting and shipping large numbers of living ticks for experimental purposes are described.

4. Observations on the seasonal trend of the tick population showed activity beginning in February and reaching a peak in late May or early June. There was little to no activity from October through January.

5. Tick-host data for June to September 1943 are tabulated. The deer is shown to be the dominant host for the adult tick. Deer, rabbit, fox, raccoon, skunk and birds frequenting thicket and ground are the principal hosts for the immature stages of the tick. The smaller rodents are shown to be relatively unimportant hosts.

6. Observations are made on the habits and behavior of the tick.

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SOME NEW ASPECTS AND APPROACHES TO THE PROBLEM OF DUST DISEASES*

CARL U. DERNEHL AND CARL A. NAU

References to industrial disease are suggested in Egyptian, Greek, and Roman writings on mines and mining. Among the hazardous conditions noted in mining was foulness of the air and the amount of dust generated by the mining process. However, it was not until many centuries had passed that the frequency of pulmonary complaints in miners became related to the dust content of the mine air. Paracelsus' pioneering study of "miners' sickness," written in 1534 and published in 1567, suggests dusts as one of several factors that might be involved. Agricola's great classic on metallurgy in 1556 also emphasized the importance of ventilation of mines.

Knowledge of the effects of dusts on the human organism accumulated slowly, but with the advent of the microscope and its application to human pathology in the 19th century, progress became more rapid. The final link in our knowledge came when mass X-ray surveys of miners, stone cutters and others exposed to mineral dusts, became possible.

The increasing industrialization of our country has led to widespread exposures to many dusts previously non-existent. The realization of these potential dangers by men interested in industrial hygiene has led to rapid recognition of toxic dusts and to steps toward their control. Oftentimes the control of exposure to dust becomes exceedingly difficult, and nowhere is this more apparent than in the case of the laborer exposed to the dust of silicon dioxide. While the many precautions taken have markedly reduced the incidence of silicosis in younger men, those employed over many years all too frequently still show evidence of this disabling disease. In addition, there is always the individual who, because of some imperfection in his nasal filtering mechanism, proves to be more susceptible to silicosis than his colleagues.

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*From the Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston, Texas. Received for publication April 16, 1945.

Various explanations have been advanced to explain the mechanism behind the formation of the silicotic nodule and the physiologic basis for the symptomatology. For many years silica was considered to be so insoluble that it was believed the presence of the sharp particles lay at the bottom of the pathology. Now it is known that quartz is very slightly soluble in water and body fluids, and opinion is shifting in favor of the solubility theory of origin. Gardner (1940) takes exception to this theory and presents much evidence to suggest that the toxicity is based on certain as yet unknown physicochemical surface phenomena. As an example: Intravenous injection of 1–3 micron silica produces immediate shock in animals, but silica must stand in contact with fluids for twelve to twenty-four hours before any solution can be demonstrated. That the shock is not embolic in origin is shown by the fact that other materials of the same size fail to produce any reaction.

The exact physiology behind the dyspnea of silicotics is hard to explain. Seldom do men die of silicosis, but rather some poorly handled complicating factor intervenes and causes death. The most prevalent explanation for the dyspnea is that pulmonary fibrosis and the presence of silicotic nodules so decrease the functional area of the lung that the respiratory rate is speeded up in an attempt to compensate for the decreased area. This theory is not acceptable to many students of the subject who point out that frequently the dyspnea is totally out of proportion to the area of lung involved in this pathologic process. A possible explanation of this discrepancy may be the presence of bronchospasm, first suspected in anesthetized guinea pigs following intratracheal instillation of colloidal silica. Filley and his coworkers (1945) present evidence which confirms the presence of bronchospasm and tends also to refute the toxicity of dissolved silica. By the use of a heart-lung preparation they were able to ascribe a definite broncho-constrictor effect to colloidal silica and to show that this constriction was not produced by colloidal gold and could be relieved by adrenalin. Conversely, the colloidal silica was able to produce bronchoconstriction at the height of a relaxation phase produced by a previous injection of adrenalin. The reaction was not due to any particulate blocking of the preparation.

Silica in solution produced no action whatsoever, even in amounts 10 times that used for the colloidal material, thus tending to refute the toxicity of dissolved silica.

Since control measures have failed completely to eradicate silicosis in persons exposed to silica dust, the search for a further means of control has not ended, and much recent work has given high promise of a speedy solution to the problem. Heffernan in 1932 suggested that the toxicity of freshly fractured silica lay in the fact that the silica, because of its molecular structure, presented many unsatisfied oxygen molecules to react with the body tissues. Based on this theory, a group of Canadians, Denny, Robson and Irwin (1937), felt that this toxicity could be destroyed by providing nascent hydrogen to satisfy the demand of the unsatisfied oxygen, thus rendering the quartz non-toxic.

Following this idea, Denny et al., added small quantities of aluminum to quartz suspensions and found that the solution of the quartz was almost completely prevented. Since Gye and Purdy (1924) demonstrated that the toxicity of silica was due to its solubility rather than to its mere physical presence, there was the distinct possibility that silica could be detoxified in the animal body by the simultaneous presence of aluminum. To test this hypothesis, Denny and his group exposed 7 rabbits to quartz dust alone. At autopsy the 6 control animals presented well developed fibrosis whereas the treated animals showed practically none. These favorable results on a small number of animals led to further studies (1940) to determine the most effective concentrations of aluminum dust and whether the aluminum dust itself would produce any pathologic change. Rabbits exposed for twelve hours daily to 198 million particles of aluminum per cubic foot of air for a period of fourteen months showed no significant changes in the lungs at autopsy. Thus, aluminum dust could be classed as non-toxic. Denny was able to produce silicosis in rabbits in five months by exposure for twelve hours a day. It was found that by the addition of 1.0 to 1.5 per cent aluminum dust to quartz dust, slight fibrosis could be produced in 3 out of 10 animals. If the percentage of aluminum dust were increased to 1.5 to 2.0 per cent, it afforded complete protection in all animals. It also

was demonstrated that rabbits could be exposed to quartz dust for twelve hour periods and then receive forty minutes' exposure to aluminum dust with complete protection of the animal, provided that the aluminum concentration in the lung was 0.5 per cent or greater, as compared to the amount of silica present. The increase in efficiency in the latter series of exposures was believed to be due to the more uniform small particle size of the aluminum dust; most particles were three microns or less in size.

The exceedingly encouraging results obtained by the exposure of animals led to detailed chemical studies to determine, if possible, the mechanism by which the aluminum exerted its protective action, as well as the length of time that this protective action could be expected to last. Denny and his coworkers found that when aluminum powder was added to water, a material was formed which had the property of causing flocculation of dispersed silica particles; this material absorbed small quantities of silica and most important, was itself adsorbed onto the surface of the silica particles. Staining with aurin dye, specific for aluminum hydroxide, demonstrated that what appeared to be aluminum hydroxide had coated the silica particle. Since aluminum hydroxide is known to be practically insoluble in a pH range from 6 to 11, it was believed that the basis of its action lay in covering the silica particle with an insoluble coat of aluminum hydroxide which would prevent the silica from going into solution. Experiments have shown that this protective action still was effective at the end of seventeen months. Studies by Germer and Storks (1939), employing spectroscopy and electron diffraction patterns, have tentatively identified the aluminum compound as a gelatinous hydrated aluminum oxide rather than aluminum hydroxide as first was believed.

Working independently of the Canadian group, Gardner, Dworski and Delahant (1944) reached similar basic conclusions, although the method of approach was different. Gardner and his group attempted to coat silica particles with an insoluble layer, thus preventing toxicity. Their first attempts to use iron hydroxide were not entirely successful and so aluminum hydroxide was chosen as a more insoluble coating.

Using their own preparation of aluminum hydroxide, they were able to demonstrate a complete protection by the intravenous injection of silica in one ear of a rabbit and simultaneous injection of aluminum hydroxide into the other ear. Then began a search for a form of hydrated alumina which could be administered by inhalation, since the first hydroxide proved effective only in its original solution and could not be dried. Such a material was developed by the Aluminum Company of America and proved to be as active on intravenous injection as was the original hydroxide. Attempts to use aluminum metal as described by Denny and his group were not successful, but this was believed to be due to the fact that the aluminum powder was old and surface oxidation probably interfered.

Gardner's work is not yet complete, but he reports the following trends: Hydrated alumina in very heavy doses may produce transient reactivation of tuberculous lesions in guinea pigs. Silicosis can be completely prevented, provided the silica and the aluminum gel can be brought together in the same phagocytic cell. Immature silicotic lesions can be caused to regress, but established fibrotic lesions are rendered quiescent, while the silicotic reaction in regional lymph nodes is not affected. The use of hydrated alumina seems to possess several advantages over aluminum metal: It does not flocculate on contact with body fluids, it is stable and need not be freshly prepared and its white color is less objectionable than the black color of finely divided aluminum.

The use of aluminum powder in the treatment of silicosis has had only a very limited trial. Gardner (1944) reported several cases with rather disappointing results. Crombie, Blaisdell and MacPherson (1944) reported the results on 34 active miners with uncomplicated silicosis and measurable pulmonary disability who received daily treatments of thirty minutes duration over a period of two hundred to three hundred days. Nineteen, or 55 per cent, of the men so treated showed clinical improvement in that there was a decrease in cough, chest pains, shortness of breath and fatigue. Fifteen, or 45 per cent, demonstrated no improvement, but neither was there any progression of their disease in spite of continued exposure to

silica. Respiratory function has improved in 12 of the 34 cases, as shown by repeated tests. A group of 9 controls, who received no aluminum therapy, showed progression of the disease in 66 per cent of the cases while under observation. Thus it would seem that aluminum dust possesses some value as a therapeutic agent in silicosis, but its greatest value undoubtedly will lie in the prevention of silicosis in those not yet affected.

For many years there has been much controversy over the question of the pulmonary lesions produced by cotton dust. Most students of the subject readily admit that workers exposed to high concentrations of cotton dust present symptoms of respiratory tract irritation. In addition, the English workers discuss a febrile episode, "Monday Fever," which frequently occurs in the card and stripper rooms. Several years ago one of the authors (C. A. N.) had the experience of being called to several cities where cotton for making mattresses was being provided for the poor. The mattresses were made by old-fashioned methods and enormous quantities of dust were created in rather confined quarters. The cotton used in each case was low grade, dirty, and slightly yellow stained. In most instances it came from areas where the bolls could not mature before frost, and where the entire boll was picked and cracked and the cotton removed. This cotton often felt "sticky." Many individuals working with this cotton suffered from severe chills and fever, chest pains, cough and generalized aches and pains. The illness lasted from two or three days to four weeks, and in some instances required hospitalization. Neal and his coworkers (1942), in studying cotton obtained from these sources, were able to demonstrate the presence of a gram positive bacillus which was capable of producing the febrile reaction. Thus it would seem that this acute febrile disease is a distinct clinical entity due to a bacterium and cannot truly be classed among the pneumoconioses.

From a wide survey of the literature, it would seem that much of the confusion regarding the toxicity of cotton dust lies in the multiplicity of etiologies. Feil (1936), in France, classed the dust as a simple organic dust which acts as a bronchial irritant and does not produce a sclerosing pulmonary lesion. Thus, emphysema and bronchial dilatation, as found in some

cases, would have their origin in the coughing effort necessary to expel the irritant dust. Feil stated that the dust never reaches the alveolus proper. In this view he is opposed by Maitland (1932) and his group who stated that small particles of cotton dust are capable of reaching the alveolus and producing there an inflammatory reaction. Prausnitz (1936) noted similar changes in experimental animals, and went on to describe a marked thickening of the alveolar wall such as would occur in interstitial pneumonitis. This latter change may well account for the dyspnea and impairment of respiratory function so often described in cases presenting no demonstrable emphysema. Nor would a normal roentgenogram be surprising since such a lesion would hardly be expected to produce any visible changes.

Work by Maitland et al., Prausnitz and Brown (1929) has shown that in certain individuals there is a definite sensitivity to cotton seed protein. Prausnitz was able to demonstrate this sensitivity in all cotton workers having respiratory complaints, while those exposed to cotton dust and having no complaints showed only rare positive reactions. Maitland also reported that the protein fraction of cotton dust is toxic on parenteral injection whereas the lipoid fraction is not. This is interesting in view of the known toxicity of gossypol, which is present in cotton seed and on the fibres of yellow stained cotton and presumably in small amounts on the fibres of white cotton.

Macdonald (1934) was able to demonstrate the presence of histamine or a histamine-like substance in cotton dust. In addition, Haworth and Macdonald demonstrated an increased amount of histamine in the blood of cotton workers, and noted that this increase was most marked on Monday mornings. The latter workers then postulated that Monday fever was due to the accumulation of histamine in the lung tissues over the week-end, and that when the worker returned to work and was exposed to cotton dust again, this histamine was passed rapidly into the blood stream in large amounts due to the presence of the allergen in the lung. There is much question as to whether this is really the mechanism behind Monday fever, but it does serve to point up the allergic reactions that may occur to cotton dust.

In summation it would seem that cotton dust is capable of producing pulmonary changes, that these changes are not the fibrotic proliferation seen in silicosis but rather a diffuse fine increase in the connective tissue framework of the alveolus, and that a sensitivity to cotton dust protein must exist to serve as a causative agent for the interstitial pneumonitis. In addition, the mere presence of the dust as a bronchial irritant may, over a span of years, produce bronchial dilitation and emphysema due to repeated coughing efforts.

An outstanding example of pneumoconiosis incidental to modern industrial methods is the case of welders siderosis. Doig and McLaughlin, in England, first reported roentgen ray changes in welders in 1936. They described a diffuse pseudonodular infiltration of the parenchyma and a marked increase in the bronchial markings and hilar shadows. There are apparently no clinical symptoms and the lesions are not debilitating. Enzer and Sander (1938) had the unique opportunity of performing an autopsy on a welder showing roentgen ray evidence of pulmonary infiltration. They described many phagocytes loaded with pigment, and also noted a tendency for perivascular and peribronchiolar concentrations of these phagocytes. There was no evidence of any connective tissue proliferation in the lung, and the opinion was that the reaction of the lung was typical of that produced by an inert dust. Chemical reactions identified the pigment material as iron, and further studies demonstrated the absence of silica in any significant quantity. A more recent paper by Sander (1944) is of interest in that one of the welders with a slight increase in trunk shadows developed minimal tuberculosis, and in spite of continued exposure to welding fumes, the lesion progressed to healing without complications. Sander's further observations suggest that lung lesions may be produced in six to ten years by excessive exposure to welding fume, especially in confined, poorly ventilated places.

Gardner and McCrum (1942) confirmed the work of Enzer and Sander. Guinea pigs exposed to welding fume showed many phagocytes heavily loaded with pigment which proved to be magnetic iron sesquioxide. However, they were not able to demonstrate the peribronchial and perivascular infiltration

described by Enzer and Sander. Gardner and McCrum confirmed the opinion of the latter workers that the roentgen ray appearance is due to the presence of iron pigment rather than to any proliferative reaction in the lung. Gardner and McCrum also demonstrated conclusively that welding fumes have no harmful effects on tuberculous lesions, and that the fumes will not reactivate old healed lesions. Although Gardner's work was done using coated welding rods containing 20 per cent silica, none of this material was present in the form of quartz.

While it would seem that the lesions as described are innocuous, there is the distinct possibility that some of the alloy welding rods with special coatings may not prove to be equally harmless. Some of these coatings contain considerable amounts of fluorides which are known to be highly irritating. Detailed studies using all types of coated welding rods are needed to determine whether other substances, such as silica, may occur in toxic form.

In a great many toxicological studies of dusts it has been common practice to expose animals by means of feeding, subcutaneous, intraperitoneal or intravenous injection. While such procedures may serve to determine the minimal lethal dosage in acute exposures, they also may be highly misleading. By far the great majority of industrial dust exposures are respiratory in origin and represent small doses over a period of years, presenting the picture of chronic rather than acute intoxication. Only by exposing experimental animals to dusts, in the same way that the exposures occur in industry, can a true evaluation of the toxicity of a dust be reached. The validity of this belief is shown in a paper soon to be published in which it will be demonstrated that inhaled antimony trioxide is many times as toxic as the same amount of the compound administered intraperitoneally. To prove such ideas, the authors (1944) have developed a dust exposure apparatus in which experimental animals can be exposed to varying controllable concentrations of dust in a manner similar to exposures as they occur in industry. Briefly, the apparatus consists of an air operated ejector mechanism to pick up and transport the dust, a cyclone to size the dust to three microns or less, an

exposure chamber provided with openings for air sampling procedures, and an electrostatic precipitator to strip the effluent air of dust. The apparatus is placed in an air conditioned room so that temperature and humidity can be adjusted as desired. It is also possible to use forced exercise of the animals when indicated. Studies of this type cannot be done in a short period of time but require exposure of a year or more to heavy concentrations of the dust being investigated. While the length of time required for such experiments is a distinct disadvantage, the greater accuracy of the end results has led to extensive plans pointed toward investigation of dust hazards as they occur in Texas industries. One of these studies has been completed and several more are now in progress.

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AMPUTATIONS IN THE UPPER THIRD OF THE LEG

G. W. N. EGGERS*

Amputations of the leg, distal to the knee and proximal to the ankle, always have proved to be successful when performed about 5 inches below the knee joint. The area has been so desirable that it is described as the "seat of election." This amputation has been very satisfactory because it permits the use of artificial support. Even the amputation with a very short tibia, in flexion, permits the use of the so-called "peg leg." So famous is this operation that it has a firm niche in romantic tales and dramatic stories.

The technique of the operations of amputation in the upper third of the leg has the formal approval of all anatomists and surgeons, whether the equal, circular or lateral flap technique, or any other sound anatomical method is employed. The length of the muscular flaps, in general, is taken at a total length of one and one-third diameters of the leg at the amputation level. The site of the saw cut of the fibula is generally more proximal than the tibia. The greater contraction of the soleus and gastrocnemius muscles requires the posterior portion of the flap at line of operation to be longer than the anterior tibial portion so as to allow for smooth, even contour of the stump. The muscular flap must not be too long.

The effort to restore some reasonable degree of function after amputation has been the ultimate goal of all surgeons. The early crude fork of a tree (Fig. 1), the more practical peg leg (Fig. 2), and the more efficient artificial leg have been of great help to unfortunate individuals. The use of prostheses has improved and been so perfected that the present trend of amputations is to perform a suitable amputation to meet the requisites of an efficient apparatus rather than to decrease the efficiency of the prosthesis to conform to the amputation. Thus, a reasonable coöperation of surgeon and mechanic produces a better functioning substitute for the patient.

*From the Department of Surgery, Orthopedic Division, The University of Texas Medical Branch, Galveston, Texas. Received for publication April 2, 1945.

Fig. 1. Fork of tree as leg support.

The most annoying feature of amputation in the upper third of the tibia, granting that other factors are satisfactory, is the presence of the fibula. This bone in the stump is freely movable with a distinct joint at the proximal end with the tibia (Fig. 3). The bone is unstable, and the head of the fibula with the encircling peroneal nerve protrudes laterally and posteriorly and prevents an even contour on the stump (Fig. 4). These facts deserve consideration in preparing for the modern artificial leg, since the weight is borne largely on the sloping surface of the tibial condyles, especially anteriorly. The receptive appartus, usually a leather cup (or similar material),

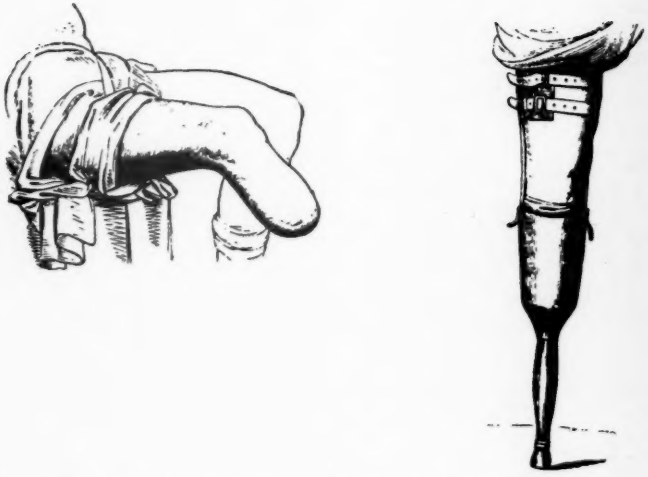

Fig. 2. Peg leg from Fergusson's Practical Surgery, 1848.

fits the stump by properly made plaster impression and mold. The socket must be so finished that it will prevent any pressure on the skin-covered head of the fibula. If there should be pressure, the prosthesis will cause pain and the patient will become very uncomfortable; many times the skin may suffer pressure necrosis as a result.

The annoying presence of the fibula has been overcome by its removal from the stump at the time of amputation. The technique is simple and the method employed is adaptable to any classic amputation. After the skin and muscle flaps are completed, the tibia is divided at the proper level. The fibula then is divided at about the level of the original skin incision, allowing it to be longer than the tibia (Fig. 5). The interosseous membrane is divided with the scalpel, as far as possible from the edge of the fibula, then a sharp, curved chisel is placed against the fibula and the interosseous membrane divided to the level of the neck of the bone. The muscles are easily stripped from the lateral side by the same type of chisel. An incision about four inches long is made just anterior to the fibular head (Fig. 6). The peroneal nerve can be identified

Fig. 3. Showing proximal tibiofibular joint.

but is posterior and thus quite safe in this incision (Fig. 7). The insertion of the biceps femoris is divided and then the accessible, long, distal end of the fibula grasped in a bone-holding forceps and rotated. As this is done, the capsule of the joint is divided. When this has been accomplished, the fibula will rotate completely and may be simply withdrawn from the stump. The peroneal nerve and anterior tibial artery are not

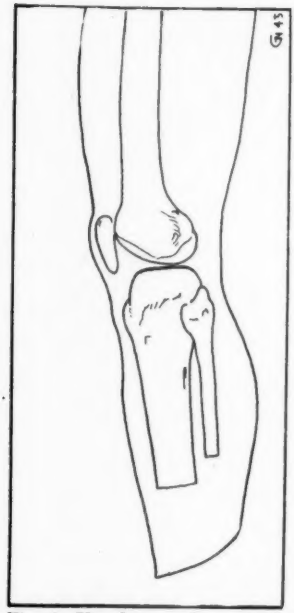

Fig. 4. Usual type of amputation in upper third of tibia.

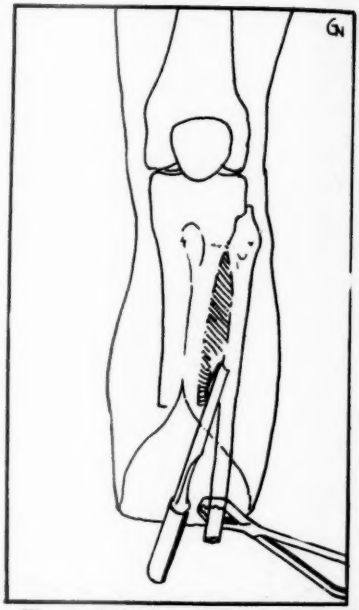

Fig. 5. Stripping shaft of fibula of interosseous membrane and muscular attachments.

disturbed. The usual closure is made after tying the nerves and injecting ether into the distal end in an endeavor to prevent the occurrance of painful stump.

The purpose of removing the fibula is to give the patient a well-formed stump which can be fitted more easily and therefore will function better in the modern prosthesis (Figs. 8, 9, 10).

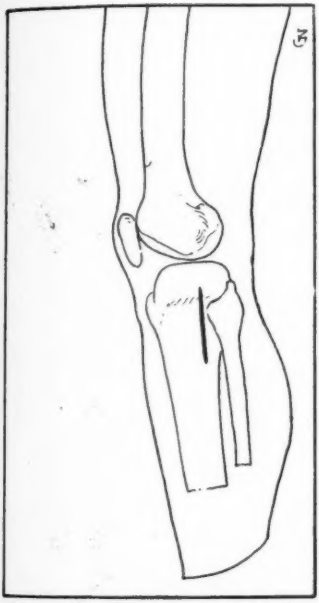

Fig. 6. Incision to expose head of fibula.

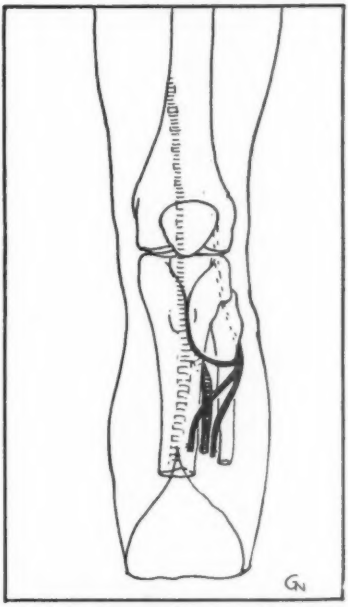

Fig. 7. Peroneal nerve and anterior tibial artery.

Fig. 8. Early stump.

Fig. 9. Showing lateral supportive contour of tibia.

Fig. 10. Prosthesis for amputation in upper third of tibia.

ORGANIC BEHAVIOR PROBLEMS*

JACK R. EWALT

The majority of the deviations in behavior of children undoubtedly are due to difficulties in adjusting the basic urges to the demands of the environment. In most instances the environmental demands are unreasonable, inconsistent or of such a nature that the child cannot establish a mutually satisfactory relationship with the world about him. It must be borne in mind that there are occasions when the difficulty lies not in the demands of the environment but in the biologic equipment available to the child for such an adjustment.

In many cases the defect is one of insufficient intellectual equipment. In these children, the behavior disorders are due to mental retardation. The behavior response is due to the child's attempt to adjust to a bewildering environment where others grasp easily things incomprehensible to him. He may adjust by (1) withdrawal, resulting in body complaints or other symptoms; (2) assault and aggressive activity, or (3) paranoid trends.

A second familiar group is the child with birth injury manifesting as secondary mental retardation and behavior disturbance. The factors mentioned above operate, and in addition, the physical handicap brings its own needs for compensation.

Another type of organic disorder which may be overlooked is the postencephalitic behavior pattern. These disorders are rather common in children who have survived an attack of encephalitis lethargica or so-called Von Economo's disease. Wilson stated that more than one-third of the patients below 16 years of age who survive the disease will show some type of behavior difficulty. Heersema and Kennedy of the Mayo Clinic followed a series of patients for an average of seventeen years and found that the patients tended to show progressive

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disturbances in behavior. Bond and Smith found that children who improved in a school environment especially adapted to children with this disorder, showed a relapse if returned to an environment unfavorable to their special problems. Holt reported progressive improvement in a ten year study at Boston Psychopathic Hospital, but Grossman, like Heersema, found the behavior symptoms progressive.

The importance of this condition is not in its frequency, but in the ease with which it is either overlooked and long and futile efforts at psychotherapy made or in which functional reactions are misdiagnosed as postencephalitic behavior disorders, and opportunities for therapy overlooked.

The symptoms of the disorder are typical and have been well described by Bender and by McDermaid. In many cases there is an absence of neurologic signs in the usual sense of tremors, paralyses, oculogyric crisis, rigidities, sensory disturbances and so forth. The typical case is remarkable for the marked motor restlessness. The child seems to be in a frenzy of activity, moving rapidly from place to place, on tables, under chairs in and out of doors, shifting his attention rapidly from one object to another as if he were trying to make contact with all of his environment at once. This type of hyperkinesia has been dubbed "organic drivenness," and once observed it is not easily confused with other forms of overactivity in children. The activity often is destructive in nature, but the destruction is of the frenzied accidental knocking over or impulsive throwing variety and not the methodical planned destructiveness seen in other types of disorder. The child is, as a rule, sickeningly affectionate one moment and an irritable, fighting, biting hellion the next.

The shifts in behavior are usually brought about by minor environmental events such as remarks by the physician, teachers or parents, and represent all-out emotional responses with little or no correlation between degree of response and importance of the precipitated situation. The patients are also very impulsive in their behavior, acting without any consideration for the consequences of their acts. As a result of this symptom, many are misdiagnosed as "constitutional psychopathic" states. Many patients in this group show some degree

of intellectual retardation, usually of the moron type, but this retardation is not universal and not necessary for the diagnosis of post-encephalitic personality change. While the disorder occurs with greatest frequency in Von Economo's disease, it also occurs in the post-infectious encephalitis cases, and in some of the severe cases of infectious chorea. As these children grow older one sees mood disorders, paranoid trends, and other alterations of psychotic proportions. Psychosomatic tests show changes typical of the organic reaction. There is usually failure in tests requiring visual or auditory memory for completion. Tests involving spatial concepts, figure reproduction or retention of body image are done poorly. The Rorschach examination reveals an organic pattern. Electroencephalographic studies usually reveal a diffuse nonspecific abnormal type of cortical rhythm similar to that seen in other forms of diffuse cortical pathology.

Typical cases are: 1. An 18-year-old boy, who developed normally until 2 years of age. At this time he had pneumonia followed by meningitis and encephalitis. Following this his intellectual and physical development were retarded. There was a reversal of sleep pattern, trouble in carrying out sustained physical effort, as in games, and slowness in movement. He adjusted poorly in school and finally was removed because of frequent fights and a fondness for lifting the dresses of little girls. He was given to outbursts of temper during which he would kick, scream and bite. Examination revealed a mild hemiparesis, the rigidity rhythmical tremor and decrease in associated movement of mild parkinsonism (Binet Simon 80). The patient was observed over a period of five years. During this time his behavior became progressively worse. He made impulsive assaults on persons, preferably female. He had become sexually aggressive, and on one occasion beat a girl with whom he just had had sexual intercourse. This behavior necessitated continuous custodial care. About one year ago a prefrontal lobotomy was performed and since then he has been less explosive and assaultive and now may be at large in the community with a companion.

2. A 14-year-old white girl was brought to the hospital because of overactivity and spells of temper. The patient was well and had developed normally until April, 1942. At this time she had an attack of measles during a local epidemic; she ran a high temperature and was in stupor for five or six days. After this attack she became unmanageable. She stamped, swore, scratched, poked at peoples' eyes and made loving advances only to bite or scratch when near. During her waking state she was in a

constant frenzy of activity. Throughout her stay in the hospital she remained unimproved.

Treatment in terms of recovery is disappointing and the most optimistic reports give low figures for improvement. Since these children have damaged mental equipment with which to adjust to their environment, the environment must be adjusted to them. This is done best in special schools where the tasks are simple, changing, and offer a minimal opportunity for distraction of attention from the task at hand. In our experience these children take to simple farm duties, simple gardening, simple tasks of construction, painting and so forth. All such activity must be supervised. In most of these cases better adjustments are made with institutional than with family care, however untiring the family may be. Drug therapy has been uniformly ineffective in our experience, although large doses of barbital may render them less active for short periods while institutional care is being arranged.

Another type of behavior response due to organic pathology is epileptic equivalent states in children. These are especially apt to be misdiagnosed as functional disorders because the routine neurologic examination yields uniformly normal findings and there is rarely a history of convulsions, fits or spasms.

Careful elicitation of the history often will reveal that the antisocial behavior usually comes in "spells" or "attacks." These children often are described as average or better in behavior the greater percentage of the time, but with short lapses of very aggressive or destructive behavior, truancy or running away. At times it is noted that the child denies any knowledge of his actions; the distracted parents often interpreted this as lying. The behavior itself may consist of short periods of confusion and at times hallucinatory experiences. The most common reaction is one of periods of great irritability, bursts of rage and destructive, and at times homocidal, behavior.

The diagnosis is dependent upon the history and the demonstration of the convulsive pattern in the electroencephalogram.

A typical case is as follows: A 10-year-old boy was brought from jail where he had been taken after he had attacked a child in school with a pop bottle. The stepmother reported that recently he had attacked her

with an ice pick, that he pulled the legs off of chickens and abused his dog. She also stated that he was not always cruel, and when on his good behavior he was like any other boy. During his spells he clenched his fists and assaulted any available living object with any weapon at hand. His stepmother believed that he had intended to kill her, and also the boy he attacked in school. He also has stolen toys and small sums of money. At one time, while in the Orphans Home, he set fire to the place, and at another time he was caught having sex relations with a small girl.

His birth and early development were normal. His father had epilepsy. His mother died of a tumor of the stomach.

Physical examination was normal. The neurologic examination was normal. The glucose tolerance curve was low, flat type

0	3 hr.	6 hr.
83	87	74

and the electroencephalogram was characteristic for a convulsive disorder. The patient was placed on Dilantin and his behavior improved; there were no further outbursts. His parents refused to accept him in the home and he was placed for adoption. Two years have elapsed and he now is living with foster parents and getting along well, but continues on medication. There have been no further aggressive outbursts to date.

As illustrated in the above case, treatment in this group is usually most satisfactory. Dilantin is the drug of choice, provided that adequate medical supervision is available. If it is not available, phenobarbital may be substituted, although in our experience it is much less effective in this particular form of epilepsy.

In considering the cause of any type of behavior problem one must consider two factors. First, the individual who is having the problem, and second, what situation he is trying to adjust to. In all instances the consideration of the individual must be thorough and must include thorough physical and psychiatric studies if errors in diagnosis are to be avoided. In the above material the primary defect is in the tissue of the reacting individual, making it difficult or impossible for him to react to ordinary life situations, and causing him to react in unusual ways in his attempts to reach an adjustment using his faulty equipment.

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PARA-AMINOBENZOIC ACID: ITS ACTION ON EXPERIMENTAL ROCKY MOUNTAIN SPOTTED FEVER

LUDWIK ANIGSTEIN AND MADERO N. BADER*

On the basis of favorable results in the treatment of rickettsial infections with p–aminobenzoic acid (Yeomans, 1944; Greiff, 1944), this member of the vitamin B complex was tried on guinea pigs infected with Rocky Mountain spotted fever of high virulence. In untreated animals a regular 72 hours' period of incubation is followed invariably by high fever of 5 to 8 days' duration. The death-rate in infected guinea pigs is 100 per cent.

The present work was directed into the possible protection of animals against the disease by the use of PABA. For this purpose guinea pigs were given the drug in proportion of 2 Gm. of the powder per each 100 Gm. of high protein feed ad lib. The administration of this mixture was started shortly after the intro-abdominal injection of the infective material (spleen or blood). In other series the drug was given 24 hours prior to the infection or 24, 48 and 72 hours thereafter. The daily PABA treatment was then continued for 5 to 7 days after which time it was withdrawn.

As compared with the clear-cut febrile and fatal disease in control animals a striking change in the response of test guinea pigs was observed. Out of 12 guinea pigs in which PABA intake preceded the infection by 24 hours, 3 animals showed fevers of one to three days' duration; 2 guinea pigs reacted with slight and late elevations in temperature for one to two days while 7 animals remained completely afebrile. Two deaths were recorded. In another series of 17 guinea pigs treated with PABA beginning with the day of spotted fever injection, 11 animals remained afebrile during two weeks' observation, 3 reacted with one or two days' fever, 2 developed typical spotted fever and died, while one died showing no

*From the Department of Preventive Medicine, The University of Texas, Medical Branch, Galveston, Texas. Received for publication April 29, 1945.

fever. As a whole, in 80 per cent of test guinea pigs the fever was either entirely absent or reduced to abortive mild attacks.

When PABA was administered during the incubation period, some of the guinea pigs remained afebrile when the drug was given 48 or 72 hours after infection. Recent experience showed, however, that the administration of PABA 24 hours following infection is apparently the longest delay of its protective role.

These facts indicate that PABA given before or shortly after infection can prevent the appearance of clinical manifestations of spotted fever. Nevertheless, some afebrile guinea pigs showed pathologic lesions (splenomegaly, pneumonitis) typical for spotted fever. When the spleen of these animals was injected into normal guinea pigs the latter reacted with typical spotted fever like the controls. The surviving afebrile animals were then reinoculated with spotted fever rickettsiae and showed complete immunity. It seems therefore that the absence or mildness of clinical symptoms indicate rather a suppressive than destructive effect of p–aminobenzoic acid on spotted fever rickettsiae with the result of inapparent (sub-clinical) infection and solid immunity.

The present study still in progress extends the previously observed effectiveness of p–aminobenzoic acid in louse-borne and murine typhus and proves its protective value in the highly virulent Rocky Mountain spotted fever.

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CALLING ATTENTION TO:

Items of Possible Interest to Friends of Our Effort

March, 1945

1. Books, Books, Books: C. C. Thomas of Springfield, Ill., comes to town again with M. G. Sevag's *Immuno-Catalysis* (preface by S. Mudd, 1945, $4), & S. E. Gould's *Trichinosis* (1945, $5). Commonwealth Fund (41 E 57, NY 22) announces publications of I. Galdston's Committee on Medicine & the Changing Order of NY Academy of Medicine: B. J. Stern's *American Medical Practice in Perspectives of a Century, & Medicine in Industry;* E. H. Corwin's *The American Hospital;* F. D. Mott's *Rural Medicine;* N. Sinai's *Varieties of American Insurance & Prepayment Plans for Medical Care;* R. B. Allen's *Medical Education;* H. S. Mustard's *The Development of Community, State & National Public Health Services;* E. J. Stieglitz's *Preventive Medicine;* A. Gelinas's *Nursing;* R. H. Shryock's *Medical Research,* & M. W. Carr's *Dentistry*. Remember the publications of the Committee on the Costs of Medical Care, which Univ. of Chicago Press published between 1928 & 1932? Excellent is H. B. Richardson's *Patients Have Families* (Commonwealth Fund, NY, 1945, $3). Also excellent is K. D. Blackfan & L. K. Diamond's *Atlas of the Blood in Children* (Commonwealth Fund, NY, 1945, $12). D. I. Abramson offers *Vascular Responses in the Extremities of Man in Health & Disease* (Univ. Chicago Press, 1945, $5). Much on USSR medicine is included in *Science in Soviet Russia* (Cattell, Lancaster, Pa., 1945, $1.50). H. C. Solomon edits *Manual of Military Neuro-psychiatry* (Saunders, Phila., 1945, $6). G. J. Heuer reviews 10 years experience on the *Treatment of Peptic Ulcer* (Lippincott, Phila., 1944, $3). And for escape there's *The Thurber Carnival* (Harper's NY, 1945, $2.75), and J. Barzun's *Teacher in America* (Little Brown, NY, 1945, $3).

2. Semiotics: W. D. Collings recommends F. C. Redlich's investigation into the use of medical terms (The Patient's Language, *Yale J. Biol. Med.*, 17:427, 1945). Also note C. D. King's study of the meaning of normal (*Ibid.*, p. 493). Univ. California Press will publish A. B. Johnson's *Philosophy of Human Knowledge, or a Treatise on Language* (NY, 1828).

3. Infections: M. Mitman well discusses aerial infections, condemning hospital blankets & overcrowded busses & faintly praising ultraviolet radiation (Brit. Med. J., 1:72, Jan. 20, '45). M. G. Sevag studies mechanism of resistance of organisms to sulfonamides, & inhibitory action of zephiran (*J. Bact.*, 48:615–638; 677, 1944). W. Heine commends serodiagnosis of Tb (*Zentr. Bakt. Infekt.*, 151:174, 1944). W. E. Coutts offers evidence indicating respiratory infection with lymphogranuloma virus (*Rev. Chilena Hyg. Prev. Med.*, 6:161, 1944). National Research Council issues mimeoed survey of antimalarial drugs by O. Temkin & E. M. Ramsey, deserving book printing (Washington, 1944, 237 references).

4. HISTAMINE: C. V. Anrep & Co. of Cairo discuss excretion of free & conjugated histamine in urine (*J. Physiol.*, 103: 155, 1944). F. Alexander describes metabolism of histamine noting high skin content & kidney excretion (*Quart. J. Exp. Physiol.*, 33:71, 1944). M. Laskowski & J. M. Lemley study histaminase, obtaining protective but toxic product (*Arch. Biochem.*, 6:105–120, 1945).

5. AND THEN: J. B. McClinton homilizes on the Doctor's Own Ethics (*Canad. Med. Asso. J.*, 52:199, 1945). D. A. Roman-Vega & J. Adriani (*Anesth. Analg.*, 23:248, 1944) confirm D. Jackson's report on sympathomimetic action of 2-methyl-amino-heptane (*J. Lab. Clin. Med.*, 29:150, 1944). I. Gersh & M. A. Still note significance of blood vessels in fat tissue in gas exchange (*J. Exp. Med.*, 81:219, 1945). A. A. P. Leao & R. S. Morison note depression of electrical activity of cortex following tetanic stimulation, similar to epiletiform discharges (*J. Neurophysiol.*, 8:33, 1945). L. Colbrook & Co. report on burns & scalds (*Med. Res. Coun. Spec. Rep.* 249, London, 1944). J. H. Annegers & Co. recommend dehydrocholic acid to prevent hepatotoxic action of arsenicals (*Arch. Derm. Syph.*, 51:112, 1945). A. S. Wiener & Co. show relation of Hr factor to Rh blood types (*J. Exp. Med.*, 81:63, 1945). W. M. Parkins & Co. report neosynephrin in gelatin solutions with penicillin prolongs blood levels for several hours after IM injection (*Science*, 101:203, Feb. 23, 1945). A. Marshak & A. C. Walker find liver chromatin (A. Claude & J. S. Potter, *J. Exp. Med.*, 77:345, 1943) stimulates mitosis and granulation in wounds, & that phosphorus building in nucleus offers way to control gene mutations (*Science*, 101, 94, Jan. 26, 1945). J. Barcroft & Co. suggest that Wharton's jelly acts as a conducting pathway from placenta to fetus (*Nature*, 154: 667, Nov. 25, 1944). E. Slater indicates inheritable tendency of families to produce children predominantly in one sex (Ann. Eugenics, 12: 121, 1944).

APRIL, 1945

1. ANTIBIOTICS: R. T. Fisk, A. G. Foord & G. Alles show prolongation of penicillin action on IM injection by adding epinephrin (*Science*, 101: 124, Feb. 2, 1945). R. L. Libby notes stability of penicillin salts in cottonseed oil suspension & relatively persisting therapeutic blood level (0.05 units/cc) from oral administration, even with impure samples (*Ibid.*, p. 178, Feb. 16, 1945). Then W. M. Parkins & Co. say gelatin with vasoconstrictor adds to prolonged penicillin level after IM injection (*Ibid.*, p. 203, Feb. 23, 1945). While C. D. Armstrong & Co. say 5% dextrose is potent prolonging vehicle for IM injection (*Proc. Soc. Exp. Biol. Med.*, 58:74, 1945). C. J. H. Little & G. Lumb recommend giving penicillin orally with egg white, after dose of antacid (Lancet, 2: 203, Feb. 17, 1945). I. N. Asheshov & F. Sterlitz isolate material from Aspergillus fumagatus active vs M. tuberculosis (*Science*, 101:119, Feb. 2, 1945). G. Schwartzman finds amino acids antagonize penicillin action vs gram-negative bacilli (*Ibid.*, 276, March 16, 1945). B. Puetzer & Co. finally synthesize clavacin (*Ibid.*, 307,

March 23, 1945). J. Charney & Co. report antacids aid gastric absorption of penicillin (*Ibid.*, 251, March 9, 1945). J. M. Bahn & Co. produce in vitro penicillin resistant gonococci with structural changes (*Proc. Soc. Exp. Biol. Med.*, 58:21, 1945). B. F. Chow & C. M. McKee think chemical inactivation of penicillin by cysteine is due to sulfhydryl & amino groups (*Ibid.*, 177). Then there's a couple little symposia on penicillin (*US Naval Med. Bull.*, 44:453–493, 1945) (*Brit. Med. J.*, 2:107–114, Jan. 27, 1945, including L. P. Garrod on activity on bacteria), and a neat new brochure by Merck. When will silly secrecy on constitution be relaxed?

2. Symposia: Fairly comprehensive on rheumatic fever, with much on control programs (*J. Pediatrics*, 26:209–264, 1945). Interesting on sterility (*Med. J. Austral.*, 2:129–145, Feb. 10, 1945). Anxious on deceleration of medical training program (*J. Asso. Amer. Med. Coll.*, 20:65–82, 1945). Anonymous, but well organized & illustrated are various War Dept. Technical Manuals, such as one on *Educational Reconditioning*. M. Fishbein edits one on *Medical Uses of Soap* (Lippincott, Phila., 1945, $3).

3. Books: E. C. Crocker's *Flavor* is intriguing (McGraw-Hill, NY 18, 1945). Blackwell's of Oxford have many new musts: H. J. B. Atkins' *After-Treatment* (18s, 2nd Ed., 1945); D. N. Matthews' *Surgery of Repair of Injury & Burns* (45s, 1945); R. R. MacIntosh's *Local Anesthesia* (10s 6d, '45). Have you the 7 experimental biology monographs issued by Macmillan's (60 5th Ave., NY 11)? E. Vasconcelos (Sao Paulo) writes best *Methods of Amputation* (Philosophical Lib., NY, 1945, $10). F. F. Chidester offers *Nutrition & Glands in Relation to Cancer* (Lee Fd. Nutritional Research, Milwaukee, 1944, $3). Harvard Press issues revised edition K. Landsteiner's classic *Specificity of Serological Reactions* (Cambridge, 1945, $5). L. H. Crisp writes *Essentials of Allergy* (Lippincott, Phila., 1945, $5). N. F. Conant's *Manuel of Clinical Mycology* looks good (Saunders, Phila., 1945, $3.50). Williams & Wilkins offers maternal health conference on *The Abortion Problem* (Balt., 1945). R. Wartenberg's *Examination of Reflexes* looks helpful (Year Book Pub., Chicago 4, 1945, $2.50). R. A. Moore's *Pathology* also looks well (Saunders, Phila., 1945, $10). A. Gesell's *The Embryology of Behavior: the Beginnings of the Human Mind* is a must (Hoeber, NY 16, 1945, $5).

4. Et Alia: Those interested in A. Aperia's important posthumous review of the kinetics of the peripheral vascular system (*Texas Rep. Biol. Med.*, 3:1, 1945) will find lots more similar material of value in the recently available *Hemodynamique et Angiocinetique* of D. M. Gomez (Paris, Hermann, 1941). E. J. Carey & Co. continue stimulating studies on ameboid motion & secretion of motor end plates in shock (*J. Neuropath. Exp. Neurol.*, 4:134, 1945). R. T. Simmons shows that most Indonesians are Rh positive (*Med. J. Austral.*, 2:108, Feb. 3, 1945). E. H. Ackerknecht tells a pertinent story in *Malaria in the Upper Mississippi Valley, 1760–1900* (*Suppl. 4, Bull. Hist. Med.*, Balt., 1945). P. O. Wolff's historical sketch of pharmacology (*Sem. Med.*, Buenos Aires, 1944) is OK, but uses old Prolegomenon (*Univ. Calif. Publ. Pharmacol.*, 1:1, 1938) without credit.

W. Ganado discusses clinical characters of pain (***Brit. Med., J.***, 2:141, Feb. 3, 1945). F. L. Meleney reviews prevention of infection in wounds and burns by use of sulfonamides (*SG&O*, 80:263, 1945). W. S. Hartroft and C. C. Macklin find lung alveoli average 0.025 sq. mm. in area (*Trans. Roy. Soc. Canada*, 3rd Ser., 37:51–81, 1944). C. A. Ross and E. J. Poth suggest traces of acrolein in oxidized cod liver oil may confer reputed antibacterial effect (*J. Lab. Clin. Med.*, 30:226, 1945).

MAY, 1945

1. THIS BOOK HITS JACK-POT: Certain to join the great English physiological immortals is A. E. Barclay, K. J. Franklin and M. M. Prichard's Barcroftian classic, *The Foetal Circulation and Cardiovascular System, and the Changes That They Undergo at Birth*, 275 superbly illustrated pages extending careful work published in part in *British Journal of Radiology* (1940 plus), and developed with cineradiography, and of course delightfully written (Blackwell, Oxford, Dec., 1944, 50s, and available from C. C. Thomas of Springfield, Ill.). Get it!

2. OTHER BOOKS: L. E. Napier offers 900 pages and 250 illustrations to *Principles and Practice of Tropical Medicine* (Macmillan, NY 11, 1945, $12.50). For the alert surgeon there are C. R. Murray's *Surgery of the Motor Skeletal System*, G. Humphries' *Surgery of the Soft Tissues*, and C. Pilcher's *Neurosurgery* (Lippincott's, Phila. 5, '45, $55). Morris Fishbein edits military hot-shots' *Doctors at War* (Dutton, NY 10, 1945, $5). Interesting is Schering Corp's *Sex Endocrinology* (Bloomfield, NJ, 1945, free). R. P. Parsons tells well of his naval hospital in the South Seas (*Mob 3*, Bobbs-Merrill, Indianapolis, 1945, $3.50). Worthy is J. R. Rees' Shaping of Psychiatry by War (Norton, NY 11, 1945, $2.5).

3. BOOKS CLIMB ANTIBIOTIC BANDWAGON: S. A. Waksman properly leads with *Microbial Antagonisms and Antibiotic Substances* (Commonwealth Fund, NY, 1945, $3.75). W. E. Herrell takes good seat with *Penicillin and Other Antibiotic Agents* (Saunders, Phila. 5, 1945, $5). J. A. Kolmer climbs high with *Penicillin Therapy, Including Tyrothricin and Other Antibiotic Therapy* (Appleton-Century, NY 1, 1945, $5). B. Sokoloff clings with unfinished Story of *Penicillin* (Ziff-Davis, NY 1945, $2). And Winthrop offers more free brochures.

4. MORE ON THE ANTIBIOTIC BANDWAGON: W. A. Randall & Co. describe method of estimating penicillin in body fluids (*Science*, 101:365, Apr. 6, 1945), while A. Fleming, D. H. Heilman and W. E. Herrill discuss micro methods for same (*Am. J. Clin. Path.*, 15:1–7, 1945). W. G. Myers and H. J. Hanson induce new strains of penicillium by neutron bombardment (*Science*, 101:357, Apr. 6, 1945). T. Hauschka & Co., in discussing mechanism of growth inhibition by hexenolactone, suggest antibiosis from reaction with SH groups essential in enzyme function (*Science*, 101:383, Apr. 13, 1945). R. D. Muir and G. Valley suggest cysteine for sterility test for penicillin (*Ibid.*, p. 390).

5. CHEMOTHERAPY: C. Wilson, G. Higgins & Co. agree that 5 Gms. methionine daily has no beneficial effect in infective hepatitis (*Brit. Med. J.*, 1:399–401, March 24, 1945). O. Schales & Co. find 1, 4 dichlor. 2. 3 dihydroxy phenazine indefinitely prevents growth of Staph. aureus in conc of 0.01% (*Arch. Biochem.*, 6:329, 1945). B. V. Patel (Bombay) finds 2-N^1 sulfanilamide-5-ethylthiazol is highly effective with no toxicity in monkey malaria (*Quart. J. Pharm. and Pharmacol.*, 17:297, Dec., 1944). M. M. Cantor and J. W. Scott successfully treat agranulocytosis with IV pyridoxine, which is necessary maturation factor (*Canad. Med. Asso. J.*, 52:368, 1945). W. Tadros (Cairo) describes estrogenic di-ethoxy-phenyl-bromethylenes which show antitumor action (*Nature*, 155:336, March 24, 1945). S. B. Ullman & Co. (Jerusalem) describe antitumor action of extracts of latex of Ficus caria (*Exp. Med. Surg.*, 3:11, 1945). A. Goth shows antibacterial properties of dicumarol (*Science*, 101:383, Apr. 13, 1945).

6. MORE: P. M. Dawson and F. A. Hellebrandt continue their interesting study on aging influence on work capacity in PMD (*Am. J. Physiol.*, 143, 420, 1945). P. O. Gravelle beautifully illustrates the artistry of chemicals with polarized light on crystal growth (*Merck Rep.*, 54:12, April, 1945). Note proceedings Central Society for Clinical Research (*J. Lab. Clin. Med.*, 30:337–394, 1945). J. H. Lawrence & Co. describe "chokes," a respiratory manifestation of aeroembolism in high altitude flying (*Ann. Int. Med.*, 22:398, 1945). T. S. Cullen offers worthy tribute to Max Brodel (1870–1941) 1st director of Hopkins famed medical illustration institute (*Bull. Med. Lib. Asso.*, 33:5, 1945). E. V. Verkhovskaya confirms effective anesthesia with 2.5 cc alcohol per Kg, in 50% conc. in 5% glucose (*Am. Rev. Soviet Med.*, 2:260, 1945). G. Antonoff discusses changes of properties of colloid mixtures with aging (*Arch. Biochem.*, 6:200, 1945). C. I. & B. P. Reed show that vitamin D exerts catalytic crystal orientation effect in bone (*Am. J. Physiol.*, 143:413, 1945). W. T. Liberton discusses time factors in electric convulsive therapy (*Yale J. Biol. Med.*, 17:571, 1945).